Mycorrhizal Inoculation in Afforestation[1]

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I. Introduction 123
II. History and Present Use of Mycorrhizal Inoculation . . 129
A. Africa 129
B. Australia and Oceania 136
C. Asia 138
D. Europe and the Soviet Union 140
E. North America 144
F. Central and South America 144
III. Technique of Inoculation 147
A. Soil Inoculum 147
B. Mycorrhizal Seedlings 152
C. Pure Cultures 155
D. Spores and Fruiting Bodies 160
IV. Introduced and Spontaneous Infection 162
A. Introduction of Exotic Trees Without Mycorrhizal Inoculation 162
B. Modes of Transportation of Mycorrhizal Infection . . 163
C. Indigenous Mycorrhizal Fungi 164
D. Introduced Mycorrhizal Fungi 167
E. Specificity and Adaptability of Mycorrhizal Fungi . . 171
V. Discussion 175
VI. Recommendations 181
A. Forest Research 181
B. Forestry Practice 183
References 185

I. Introduction

Mycorrhizae, symbiotic associations between fungal hyphae and roots of higher plants, were already recognized in the nineteenth century, and

[1] This review is based partly on literature studies and partly on a worldwide study tour made in 1967 covering 22 different countries. The author wishes to thank the Food and Agriculture Organization of the United Nations for financing the tour and numerous research workers and practicing foresters for valuable personal com-

mycotrophy has been intensively studied for more than 80 years. The advance of mycorrhizal research and the theories put forward during past decades have been comprehensively reviewed in a number of study reports and textbooks (e.g., Rayner, 1927; Hatch, 1937; Kelley, 1950; Bergemann, 1953; Harley, 1959; Lobanow, 1960; Meyer, 1966); consequently, a detailed literature review is unnecessary here. It will be enough to recall a few basic features of the phenomenon.

Frank (1885), the great pioneer of mycorrhizal research, already distinguished mycorrhizae of two basically different morphological types, ectotrophic and endotrophic. Endotrophic mycorrhizae are widely distributed throughout the plant kingdom and include a great variety of anatomical structures. Thus, the endotrophic mycorrhizae in the plant families Orchidaceae, Graminaceae, and Ericaceae, and in several broad-leaved trees, have different anatomical structure and probably also differ in their physiological functions.

The ectotrophic type, on the other hand, has much more limited distribution and greater morphological uniformity. It is most characteristic of the families Pinaceae, Fagaceae, and Betulaceae, which include the principal tree species of the cool and temperate forests. Recently, ectotrophic mycorrhizae have been found in trees of some other areas too, as in the Australian eucalypts (Chilvers and Pryor, 1965; and others) and several tropical species of Cesalpiniaceae (Peyronel and Fassi, 1957, 1960; Fassi and Fontana, 1961, 1962). Moser (1967) recently published the following (p. 125) list of plant genera in which ectotrophic mycorrhizae have been found.

The family Dipterocarpaceae should be added to the list (K. G. Singh, 1966), as well as the genera *Leptospermum* (Myrtaceae) (McNabb, 1968) and *Pomaderris* (Rhamnaceae) (Horak, 1968).

It is noteworthy that all the trees of the family Cupressaceae ordinarily have endotrophic mycorrhizae, and that in *Juniperus* ectotrophic mycorrhizae occur only exceptionally. The same also applies to Rosaceae and Ericaceae in the above list. Thus plants with ectotrophic mycorrhizae are a fairly uniform ecological group, being forest trees mainly of cool and temperate zones. Although endotrophic mycorrhizae are common in nature, whereas the ectotrophic type is rather exceptional, the latter has received much more emphasis in forestry research and practice because of its great prevalence on the trees that are economically most important.

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munications. A detailed report of the study tour has been delivered to FAO (Mikola, 1968).

Pinaceae:
- *Abies*
- *Keteleeria*
- *Pseudotsuga*
- *Tsuga*
- *Picea*
- *Pseudolarix*
- *Larix*
- *Cedrus*
- *Cathaya*
- *Pinus*

Cupressaceae:
- *Juniperus*

Fagaceae:
- *Fagus*
- *Trigonobalanus*
- *Nothofagus*
- *Castanea*
- *Castanopsis*
- *Lithocarpus*
- *Pasania*
- *Quercus*

Betulaceae:
- *Alnus*
- *Betula*
- *Corylus*
- *Carpinus*
- *Ostrya*
- *Ostryopsis*

Salicaceae:
- *Salix*
- *Populus*

Rosaceae:
- (*Dryas*)
- *Pyrus*
- *Malus*
- (*Crataegus*)

Ccsalpiniaceae:
- *Gilbertiodendron*
- *Brachystegia*
- *Afzelia*
- *Monopetalanthus*
- *Anthonotha*
- *Paramacrolobium*
- *Julbernardia*

Myrtaceae:
- *Eucalyptus*

Tiliaceae:
- *Tilia*

Ericaceae:
- (*Vaccinium*)
- (*Arctostaphylos*)

The fungal partner of an ectotrophic mycorrhiza is usually a basidiomycete. Some 80 species of Basidiomycetes have been expcrimentally demonstrated to be, and several times as many are suspected of being, mycorrhiza-formers with forest trees (Trappe, 1962a). There are also mycorrhizal fungi the taxonomic position of which is not known; for example, the common black mycorrhiza fungus *Cenococcum graniforme* (Lihnell, 1942) and the fungal partner of the ectendotrophic mycorrhiza in nurseries (Mikola, 1965). Recent papers have even reported some ascomycetes (*Tuber* spp.) to be ectotrophic symbionts of tree roots (Trappe, 1962a; Fassi, 1967).

Whatever the physiological function and ecological importance of the cctotrophic mycorrhizal association, the plain fact is that tree roots in natural forests are almost invariably mycorrhizal, at least in the cool

and temperate zones. Although nonmycorrhizal seedlings of pines or oaks can be grown under experimental conditions, they can hardly be found in nature. Since mycorrhizal association is almost universally distributed in the cool and temperate forests, it must be a result or a long evolution and belong to the normal life of the particular trees and ecosystems in question. Thus, Singer (1963a), for instance, compares the "ectotrophs," i.e., trees and their ectotrophic fungal associates, with lichens, in which nonchloropyllous fungi likewise live in organized symbiosis with green plants.

Singer and Morello (1960) correlate the geographic distribution of the ectotrophs with the climate. They consider moisture to be the most decisive ecological factor, especially at the early stage of tree growth, and that the fungal partner improves the water uptake of the trees and thus helps the young seedings to survive the critical stage when the danger of drought is greatest. Moser (1967) particularly points out that almost everywhere in the world both arctic and alpine timber lines are formed by ectotrophic tree species, and suggests that symbiosis with fungi enables the trees, during the short growing season, to obtain sufficient amounts of all nutrients to produce drought- and frost-resistant young shoots and thus to survive the unfavorable period of the year. The superiority of ectotrophic trees to endotrophic or nonmycorrhizal ones in obtaining water and nutrients has been proved by several experimental studies under controlled conditions (e.g., Hatch, 1937).

The above hypothesis may be a satisfactory explanation for the prevalence of the ectotrophic type of mycorrhiza in the forests of cool and temperate zones, which include the economically most important forests of the world. There are several reasons for presuming that the advantage of the mycotrophic type of nutrition is particularly accentuated in cold climates. Mycotrophic nutrition, however, is not restricted to any particular climatic areas; it is also closely related to the taxonomy of the trees. Thus, all the tropical and subtropical pine species have ectotrophic mycorrhizae like the pines of temperate zones, and the same probably applies to subtropical oaks. The families Pinaceae and Fagaceae are everywhere ectotrophic. The recent discovery of ectotrophic mycorrhiza in tropical Cesalpiniaceae as well as its wide distribution among subtropical eucalypts deserves special attention. These trees grow mainly in areas with a very pronounced dry season, where the mycorrhizal habit may confer some advantage; nevertheless ectotrophic mycorrhizae are also known in typical rain forest species of Cesalpiniaceae (Fassi, 1963). On the other hand, dry savanna forests contain a great number of trees of other families which have endotrophic mycorrhizae or are nonmy-

corrhizal. On the whole, very little is known concerning the role of mycotrophy in the nutrition of tropical and subtropical forests.

In regard to mycotrophy, Lobanow (1960) divided plants into three groups: highly mycotrophic, weakly mycotrophic, and nonmycotrophic. The first group includes, among others, the family Pinaceae as a whole and the genera *Quercus* and *Fagus*. In this group evolution has advanced furthest, possibly to obligate mycotrophic nutrition.

Mycorrhizae require little attention in the management of natural forests. Mycorrhiza-forming fungi are practically ubiquitous, and mycorrhizae are in all probability formed by the species best suited to the prevailing conditions. There is no need for the introduction of mycorrhizal fungi into the soils, and if new species are introduced, they seem to have few chances to survive in competition with the indigenous fungal population. Therefore, "99 per cent of all practising foresters will not have to lose any sleep over the problem of mycorrhizal infection" (Wilde, 1944).

In man-made forests the situation is different. Trees are often grown far outside of their natural range, and even on other continents, where the appropriate mycorrhizal fungi may be lacking. If the introduced tree species are highly mycotrophic, afforestations may fail completely simply owing to the absence of proper symbionts of the trees. This was noticed in early trials, when pines were introduced to new areas. Unsuccessful species trials may likewise result in erroneous conclusions if the trees are not provided with their natural symbionts. Thus, the story of the introduction of exotic pines to many countries begins with a long succession of failures, until mycorrhizal infection occurred in one way or another. Around 1930 such reports arrived almost simultaneously from several sources, as has been excellently summarized by Hatch (1936) and others. Thereupon, the Empire Forestry Institute at Oxford, through a questionnaire, collected information on existing experience and current practice of mycorrhizal inoculation (Rayner, 1938).

A somewhat similar situation may arise even within the natural range of a tree species if the natural conditions are drastically altered by human activity. Thus, for instance, conditions in forest nurseries differ greatly from those prevailing in natural forests, and this may cause disturbances when seedlings are transferred from the nursery to the field. Some modern silvicultural practices, such as drainage, fertilization, and prescribed burning, drastically change the environmental conditions in the soil and, consequently, may affect the delicate relationships between roots and fungi.

When large-scale afforestations were started in formerly treeless areas,

the importance of mycorrhizal research became obvious. The objectives of this practically oriented research were clearly defined by Hatch (1936): "Since it is known that different species of mycorrhizal fungi vary in their ability (1) to produce mycorrhizae with different trees, (2) to survive in different habitats, and (3) to stimulate tree growth, the early accumulation of field data on these variables is of vital importance to the success of afforestation projects."

Since these words were written, afforestation activity has grown tremendously, especially in the last 20 years. The FAO Secretariat (1967) has estimated that there are already some 80 million hectares of man-made forests in the world, and in the future some 4 million hectares will be planted every year. The bulk of this planting program involves exotic conifers, mainly pines.

Mycorrhizal research, however, has not kept pace with the rapidity of practical developments. Twenty years after Hatch made the above statement, an FAO report (Parry, 1956) had to admit: "The plain fact is that nothing is known for certain as yet." Afforestations have been successful in general, it is true, but certainly many failures could have been avoided if more information on the biology of mycorrhizal fungi and the ecology of their symbiotic association had been available. The task that was outlined 30 years ago for mycorrhizal research is today even more pressing: "The obivous need today is for precise information on the influence of different species of mycorrhizal fungi upon the growth of our most important trees planted in a wide variety of habitats. The problem, therefore, is not one which can be solved by one or even several individuals. It requires local and international study by many organizations engaged in forestation. . . . It may require many years for them to gain the information necessary to achieve the greatest benefits but this knowledge once gained may prove more useful in forestry than has a similar knowledge of the root nodule habit of legumes in agriculture" (Hatch, 1937, p. 150).

The purposes of the present review are (1) to survey written reports and field experiences on the effect of mycorrhizal inoculation; and (2) to study the present extent and applicable techniques of mycorrhizal inoculation in world forestry. On the basis of this survey, it is aimed (1) to give recommendations for an appropriate technique of mycorrhizal inoculation, where necessary; and (2) to outline a program of research for filling the most serious gaps in our present knowledge.

Because pines are by far the most important trees of exotic plantations, this review is concentrated mainly on pines. Experiences with

mycorrhizal inoculation mostly concern pines, and the few really scientific experiments have also been made with pines. Results with pines are probably applicable to other trees of the same family and, to some extent, also to broad-leaved trees with ectotrophic mycorrhizae.

II. History and Present Use of Mycorrhizal Inoculation

A. Africa

According to Gibson (1963), the first trial introductions of pines into **Kenya** were made in 1902. These and some subsequent attempts failed completely, until in 1910, on the advice of Kew Gardens, mycorrhizal soil inoculum was brought from a South African pine plantation and resulted in thrifty growth of a few individuals of *Pinus radiata*. Another successful attempt was made in 1926, when both seed of *P. patula* and mycorrhizal inoculum (soil from a *P. radiata* stand) were brought from South Africa.

These early observations clearly showed the necessity of soil inoculation for successful raising of pines in the nursery. Until 1946, however, afforestation took place on a small scale only, mainly comprising occasional species trials. Large-scale afforestation was started in Kenya in 1946, since when it has been continuing on an expanding scale. Numerous forest nurseries have been established, and when pines have been grown, soil inoculation has been the rule. Official instructions for nursery practice (May, 1953; Waterer, 1957) have also required regular application of soil inoculation.

As far as is known, there has been no new importation of mycorrhizal inoculum since 1926, but soil from successful plantations or older nurseries has been used to inoculate new nurseries. More recent experience has shown that the soil of old nurseries is now thoroughly infected with mycorrhizal fungi, and therefore annual introduction of new inoculum is no longer necessary. Experiments carried out by the East African Agricultural and Forestry Research Organization (E.A.A.F.R.O.) (Griffith and Howland, 1958; Griffith *et al.*, 1962) also showed that introduction of fresh inoculum to a previously infected nursery has no effect and that different species of pine show no preference for any particular type of inoculum.

Regular application of mycorrhizal soil as inoculum was discontinued in about 1960 in the nursery of E.A.A.F.R.O. at Muguga. However, in six nurseries which I visited in Kenya, the original instructions were still followed in 1967 and "pine soil," i.e., topsoil from a pine plantation, was

added to the soil mixture for Swaziland beds or transplant trays when pines were raised. For cypress the soil was not inoculated. The older of these nurseries were already 13 to 17 years old.

According to Streets (1962), the first attempts to introduce pines (*Pinus radiata*) into **Tanzania** (formerly Tanganyika) were made in 1900. All the early trials failed, however. The first successful plantation, a *P. radiata* stand in Lushoto, in the West Usambara Mountains, dates from 1928. It is not known whether the seedlings were inoculated or where the inoculum came from. Since, however, the importance of inoculation was already known in Kenya, the seedlings were probably inoculated, and in all probability the inoculum also came from Kenya. In response to the inquiry of Rayner (1938) a positive, although not conclusive, effect of mycorrhizal inoculation was reported from Tanganyika.

Soil of this first successful *Pinus radiata* plantation was then used to inoculate nurseries in the Usambaras and elsewhere in Tanganyika. Thus, when the first pine nurseries were established in the Kilimanjaro area in 1952, soil inoculum was brought in bags from the Lushoto stand.

When softwood plantations were started in the Southern Highlands of Tanganyika in the early 1950's, two bags of soil inoculum were brought from Southern Rhodesia (Eggeling, 1967) and nurseries were inoculated. According to Parry (1953), mycorrhizal inoculation is essential in pine nurseries, and great difficulties have been experienced in obtaining sufficient amounts of inoculum for nurseries when afforestation has been started in new areas.

Thus, nursery soil inoculation has been a regular practice in Tanganyika. Nowadays, seedlings are mainly raised in Swaziland beds and 10 to 50% of pine soil is added to the bed mixture (British Commonwealth Forestry Conference, 1962). Once the soil has become well infected, however, the use of pine soil is no longer necessary (Procter, 1967). Of the nurseries I visited in Tanganyika in 1967, pine soil application had recently been discontinued in five, but in seven others inoculation was still practiced every year. Losses may result if just a part of a new nursery is left without inoculation (Fig. 1).

In the experience of African nurserymen, a slight irregularity in the size and color of seedlings, in spite of inoculation, is a rather common phenomenon in the first year of a new nursery. This irregularity may be due to incomplete mycorrhizal infection of the soil and it disappears in subsequent years.

In **Uganda,** experiments with exotic pines were started more recently. According to Streets (1962), there were some trials with *Pinus radiata* and *P. patula* in 1910, 1925 and 1930, but probably none survived. In

1946 a forest nursery was founded at Kampala, and a bag of pine soil was brought to this nursery from Kenya; since then pines have always been raised in inoculated soil. When new nurseries have been founded, soil inoculum has been brought from Kampala nursery or from established plantations. There probably has been no further import of mycorrhizal inoculum to Uganda since 1946.

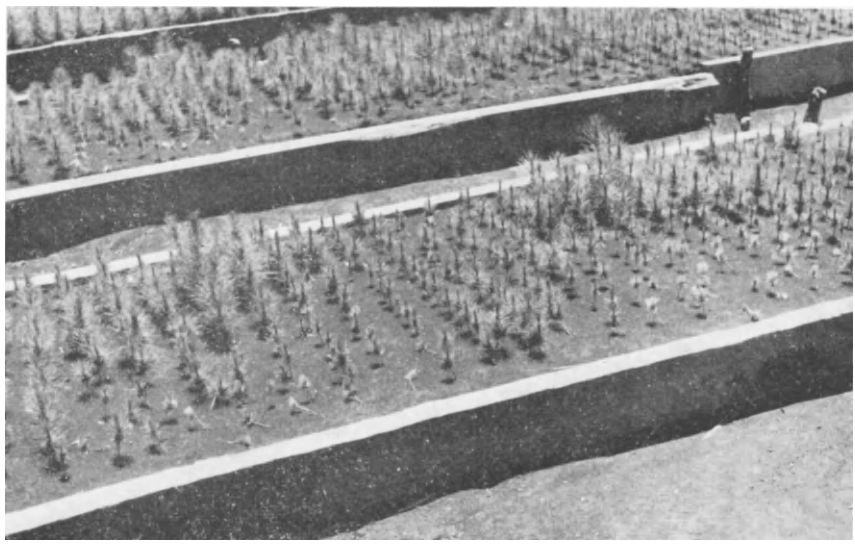

FIG. 1. *Pinus patula* seedlings growing in uninoculated Swaziland beds. Some of the seedlings have become infected and the infection is spreading out from these centers, as indicated by the improved growth and vigor of the seedlings. Uninfected seedlings are stunted and chlorotic. The sporadic infection has probably come from the inoculated section of the same nursery. Later, the infection gradually spread throughout the beds, and all the seedlings assumed a green color and rapid growth. (Londorossi nursery, Tanzania; photograph taken February 27, 1967.)

Nursery technique in Uganda was adopted as such from Kenya and Tanganyika (Stuart-Smith, 1953). In the 1950's soil mixture for transplant trays or Swaziland beds always contained 10 to 20% of pine soil, according to the instructions of E.A.A.F.R.O. By the end of the decade, however, it was found that pine soil was no longer necessary in old nurseries and its application was discontinued (Dawkins, 1967). Thus, pine soil was not used in any of the six nurseries I visited in Uganda in 1967. Today, the Swaziland technique is mainly used in Ugandan nurseries, and when seedlings are lifted, about one-third of the soil remains in the

bed; this soil is then mixed with the new soil, and this is apparently sufficient to infect the transplanted seedlings. When new nurseries are established they are always inoculated. A single application of pine soil in the first year seems to be sufficient. Some instances are known, however, in which uneven growth in the transplant beds can be attributed to insufficient mycorrhizal infection, namely, when a nursery has been extended without due care to bring infection to the new section.

Pinus radiata was introduced into **Rhodesia** from South Africa about 1900 and several thriving small plantations were established in the following decades (Streets, 1962). However, when large-scale afforestation was commenced in 1928 and nurseries were started in new areas, the necessity of soil inoculation became apparent (Anonymous, 1931). Since then, inoculation of nursery beds has been a common practice, as is indicated by official nursery instructions (e.g., Southern Rhodesia Forestry Commission, 1959).

Experiences in **Malawi** (former Nyasaland) are excellently documented by Clements (1938, 1941). Introduction of pines had been attempted since 1907, with consistent failure. Streets (1962) likewise reported that an introduction of *Pinus patula* in 1923 failed owing to deficiency of mycorrhiza. Then, in December of 1930, the remarkable results of soil inoculation in Rhodesia were reported in a private letter, which also contained an offer to send inoculated soil to Nyasaland. This offer was accepted and a parcel of soil received from Rhodesia was immediately applied to some miserable-looking pine seedlings, which in due course recovered and started good growth. This is the only known importation of mycorrhizal soil inoculum to Nyasaland. There were, however, some older pines at Blantyre Mission in Nyasaland, which had probably been brought in as potted plants, and soil from under these trees was also used as inoculum. This inoculum had the same effect as the soil imported from Rhodesia—the inoculated seedlings recovered and started to grow, whereas all the uninoculated seedlings died. Soil from these salvaged plantations was then applied to other trial plots and all the nurseries, and since then there has been no serious difficulty in growing pines in Malawi. When seedlings are grown in trays or Swaziland beds, 10% of pine soil is added to the soil mixture (Howard, 1956). The positive experience of soil inoculation was also included in the report of Rayner (1938).

Today, pine seedlings are usually raised in polythene tubes, and it is customary to add about 1 inch of pine soil to the top of the tubes (Foot, 1967). As in East African countries, there is already some indication that

pine seedlings can grow and become infected even without inoculation, i.e., infection spreads rapidly from existing pine plantations (Anonymous, 1947a).

Zambia (former Northern Rhodesia) was not so fortunate in obtaining mycorrhizal inoculum, as is indicated by correspondence preserved at the Forest Research Institute in Kitwe. At the request of the Regional Forest Office, two bags of mycorrhizal soil were sent in 1931 from Southern Rhodesia to Ndola, but, because of a foot-and-mouth disease epidemic in Southern Rhodesia at that time, the soil had to be destroyed by fire. In the following year a request for soil importation was again refused by veterinary authorities for the same reason. It was not until 1935 that an import to Dola Hill nursery was permitted, and in 1937 C. E. Duff was able to report to the Empire Forestry Institute on the "miraculous" effect of the inoculation (Rayner, 1938). [A photograph of one of Duff's experiments has been reproduced in several textbooks, e.g., Rayner and Neilson-Jones (1944) and Kelley (1950).] According to the above-mentioned correspondence, there had also been some earlier importation of soil from Southern Rhodesia to Luanshya nursery.

The principal pine nurseries of Zambia—Chichele, Chati, and Sanfya —were inoculated with soil from Dola Hill nursery, and until 1962 new pine soil was brought to the nurseries every year. Annual inoculation was then discontinued because the nursery sites had been well infected with mycorrhizal fungi and seedlings developed profuse mycorrhizae without inoculation. Mycorrhizal inoculation is not practiced in the Zambian nurseries today, and the most recent nursery instructions (Allan and Endean, 1966) do not even mention it.

South Africa has a long tradition of growing pines and other exotic trees. Donald (1965) refers to old records, according to which nurseries already existed there in the seventeenth century and large amounts of seed of pines, oaks, and other trees were imported from the Netherlands and elsewhere. There are no records of any intentional introduction of mycorrhizal inoculum; on the contrary, in a private letter of 1931 (R. P. Stephens, 1931) it is stated that "this department has never had occasion to experiment with tree mycorrhizas, as no trouble that could be ascribed to the absence of these fungi has so far been experienced with the raising of trees in plantations in the Union." Neither do historical surveys of pine introduction (King, 1925; Kotze, 1926) or of nursery practice (Donald, 1965) include any reference to mycorrhizal inoculation.

According to other sources, however, mycorrhizal inoculation has been practiced in South Africa, particularly when afforestation has been

started in new areas. King (1934) recommended application of pine soil to new nurseries in Natal, and according to Parry (1952), inoculation has generally been practiced in Swaziland.

With regard to **Swaziland,** I was able to obtain more information during my visit in 1967. When large-scale afforestation there was started by Peak Timbers Ltd. in 1948, the first nurseries were inoculated with soil from a pine plantation that had been planted in 1940 with seedlings from a nursery in Transvaal (Long, 1967). Since then soil inoculation has been practiced regularly in the nurseries of the company; soil mixture for Swaziland beds always contains some 30% of pine soil.

When Usutu Plantations were started in 1950, 3 tons of soil inoculum was brought from an old pine plantation (planted in 1930) in Transvaal and was used to inoculate the first four nurseries (Keet, 1967). Soil from these nurseries was then used when new nurseries were started. Soil inoculation has been continuously practiced in all the nurseries of Usutu Pulp Ltd., although in slightly different ways. Thus, in one of the four nurseries I visited, the soil mixture contained 10% of pine soil, whereas in the other three all the soil for Swaziland beds or polythene tubes was taken from surrounding pine plantations.

There are also some South African references to experiments with eucalypts. Both beneficial (Palmer and Pitman, 1961) and harmful (Anonymous, 1964) effects of mycorrhizal inoculation have been reported.

With regard to West Africa, most information is available from **Nigeria.** The introduction of pines and mycorrhizal fungi to Nigeria is well documented by Madu (1967). The few experiments made with exotic pines before 1950 failed completely. The first successful trials date from 1950 and 1951, when seed of three pine species were sown at Jos nursery. They were inoculated in the nursery in May of 1952 with two soil samples sent by air mail from England—from Bagley Wood (Kennington) near Oxford and from Wareham—and were planted out at Vom arboretum in 1954. *Pinus oocarpa* grew very well, whereas the climate or the soil was unsuitable for *P. canariensis* and *P. halepensis.* Soil from this small *P. oocarpa* plantation has been taken in subsequent years to inoculate pine seedlings in nurseries.

Another importation of mycorrhizal inoculum took place in 1954, when soil was brought from a *Pinus patula* plantation in Bamenda, Southern Cameroons, and a batch of 6-months-old *P. patula* and *P. radiata* seedlings was inoculated in Jos nursery. The inoculated seedlings grew well and were planted out in Vom arboretum, whereas all the

uninoculated control seedlings died. Soil inoculum from the same Bamenda plantation was brought to Jos nursery for the second time in 1956 (Wimbush, 1967).

A third source of mycorrhizal inoculum to Nigeria is Dola Hill, Zambia, from where a bag of soil was brought in 1959 and applied immediately to 1-year-old *Pinus khasya* seedlings at Jos nursery. These seedlings were planted out in 1961 at Miango near Jos, along with other pine species inoculated with soil from Vom arboretum. The Miango plantation is one of the most successful pine trials in Nigeria, and soil from this plantation, probably containing mycorrhizal inoculum from three different sources, has been used for inoculation in Naraguta nursery, where seedlings for experimental pine plantations in Nigeria have mainly been raised (Kemp, 1967). The same soil was used to inoculate the nursery of the Federal Forest Research Institute at Ibadan.

The necessity for mycorrhizal inoculation was also clearly demonstrated by an experiment at the latter Institute at Ibadan (Olatoye, 1966). Seedlings of five species of pine were raised in steam-sterilized soil, and soils from pine and cypress plantations and indigenous woodlands were used as inocula. Only seedlings inoculated with soil from pine plantations developed mycorrhizae and were healthy and vigorous, whereas both uninoculated control seedlings and those inoculated with soil from a cypress plantation or indigenous broad-leaved forests became moribund and chlorotic and had no mycorrhizae.

Personal communications (Brewer, 1967; Lamb, 1967; Fassi, 1969) on the introduction or use of mycorrhizal inoculation have also been received from **Gambia, Senegal, Sierra Leone,** and **Ivory Coast.** Palmer and Pitman (1961) mentioned that when pine seed was purchased from South Africa for the **Congo,** the buyers usually requested a tin of soil from plantations of the same species, to be used for inoculation.

In **North Africa** mycorrhizal inoculation is not practiced on a field scale. There are several indigenous species of *Pinus*, *Cedrus*, and *Quercus*. These are ectotrophic trees, and apparently soils are naturally infected with mycorrhizal fungi or the air carries sufficient numbers of spores to nurseries and new plantations.

There is, however, some indication that even in North Africa mycorrhizal deficiencies may exist or that the success of afforestation could be improved by inoculation. Thus, the uneven regeneration of *Cedrus atlantica* in Morocco has been attributed to some difficulties in mycorrhizal development (Lepoutre, 1964).

B. Australia and Oceania

The first published reports on the beneficial effect of mycorrhizal inoculation came from **Australia** (Kessell, 1923, 1927; summarized also by Bowen, 1966). Large-scale afforestation with pines (*Pinus pinaster* and *P. radiata*) was started in Western Australia in the early 1920's, and 14 new nurseries were established. The seedlings, however, grew poorly in the new nurseries and were stunted and nonmycorrhizal, and neither watering nor fertilization was of any avail. Some nurseries were even abandoned. Then the observation was made that introduction of soil from old nurseries or established pine plantations caused a complete change: seedlings assumed a healthy green color, grew rapidly, and developed numerous mycorrhizae. Although experimental proof was missing, Kessell (1927) suggested that the presence of mycorrhizal fungi was probably the effective factor in the soil inoculum.

This early experience and also a controlled field experiment of 1925 led to the following practical recommendation: "Experimental work has now shown conclusively that the only method of raising satisfactory planting stock of exotic conifers in Western Australia in a new nursery site is first to infect the soil either by applying a light dressing of soil from an old nursery or by transplanting seedling pines from an old nursery and holding them a year in nursery lines" (Kessell, 1927).

Since then it has been customary to inoculate new nurseries, especially when afforestation is extended to new areas (Kessell and Stoate, 1938), and great emphasis is placed on the proper development of mycorrhizae on nursery-grown seedlings (e.g., Adams, 1951).

Today, in areas where pines have been growing for a long time, such as the Mt. Gambier area in South Australia, inoculation of new nurseries is unnecessary and it is no longer practiced. On the other hand, when nurseries are established far from existing plantations, the soil is usually inoculated. (A practicing forester in Southern Queensland said to me: "We knew already 40 years ago that pines do not grow in nurseries without soil inoculation.") When the first pines were raised in North Queensland in about 1950, soil inoculum was brought from Southern Queensland, whereas pine trials in Northern Territory were started in 1961 by bringing mycorrhizal pine seedlings in pots from Queensland (Lamb, 1966). According to another source (Stahl, 1967), soil inoculum has also been brought to Northern Territory from the Australian Capital Territory.

There are few data available on the introduction or use of mycorrhizal inoculation in **New Zealand.** Pines were introduced there on a relatively large scale more than 100 years ago, and large numbers of seedlings were imported (Streets, 1962); thus, a widespread infection was probably present at the beginning of the large afforestation boom in the 1920's. Nevertheless, new nurseries were generally inoculated (Rawlings, 1950). According to oral information, serious trouble appeared in many nurseries and was overcome by soil inoculation. Similar reports were sent to Rayner (1938), although the evidence was not quite conclusive. Afforestation by direct seeding was also tried, in general with poor success, and mycorrhizal deficiency has been suggested as the reason for the failures.

Today, mycorrhizal inoculation is not practiced in New Zealand. Forest nurseries are often surrounded by pine plantations or are on cleared sites of former plantations; thus, a good source of infection is usually present. In some cases, however, too heavy an application of a soil disinfectant (methyl bromide) has destroyed mycorrhizal fungi and resulted in uneven and stunted growth of seedlings. This condition has been corrected by soil inoculation (Bassett, 1967).

Mycorrhizal deficiency has also been suspected as the cause of chlorosis and poor growth of Douglas-fir seedlings in some nurseries and plantations, and this hypothesis is supported by inoculation experiments (Gilmour, 1958). In general, the growth of spruce and Douglas-fir in Australia and New Zealand has not corresponded to expectations, and the role of mycorrhizae in this connection has been suggested. There always remains the possibility that the most efficient symbionts of these species are still absent, whereas the best symbionts are available to pine and larch (Pryor, 1958; Rawlings, 1958). As far as is known, there has been no intentional introduction of mycorrhizal inoculum to New Zealand from indigenous spruce or Douglas-fir forests.

Mycorrhizal inoculum, in the form of either soil or potted seedlings, has been sent from Australia and New Zealand to several Oceanian islands. Thus, pine trials were started in **Fiji** in 1947 by bringing mycorrhizal *Pinus patula* seedlings from New Zealand, and pines are now raised there in inoculated nurseries (Streets, 1962). In 1952, mycorrhizal soil was also sent from Northern Queensland to Fiji and **Tonga** (Gardner, 1967).

Soil inoculum for pine trials in **New Guinea** was brought from Queensland (J. K. White, 1967). When a new nursery was started at Kauli in 1966, both soil inoculum and mycorrhizal seedlings were brought

in from an older nursery at Bulolo, which, in turn, had been inoculated with soil from Queensland.

C. Asia

Several early reports on the effect of mycorrhizal inoculation have come from Southeast Asia. In **Java** and **Sumatra** (Roeloffs, 1930) dying and chlorotic nursery stock was saved and revived by interplanting mycorrhizal pine seedlings in the nursery beds. The same method has since then been standard practice in new and sometimes even in old nurseries (Becking, 1950; van Alphen de Veer *et al.*, 1954; Cooling, 1967). The spread of fungal infection from the mycorrhizal "mother trees" to neighboring seedlings is easily visible because of the color change and improved growth of the latter.

In the **Philippines** (Oliveros, 1932) raising of *Pinus insularis*, which is indigenous in the mountains, was tried for 20 years in a lowland nursery, always without success. The seed germinated well, but after a few months the seedlings turned yellow and died. It was assumed that the climate at lower elevations was unsuitable for the species, but, on the other hand, seedlings raised in a mountain nursery within the natural range of the species and then transplanted to the lowlands grew well. Subsequently the lowland nursery was inoculated with soil from an established plantation. Since then there has been no difficulty in raising pine seedlings. According to Oliveros (1932), inoculation of new nurseries has been practiced in the Philippines since 1919, and it is still practiced in the same way (Lamb, 1966).

In **Malaya,** mycorrhizal deficiency has not caused any serious trouble. According to Freezaillah (1967) and Lamb (1967), the first pines were brought to Malaya as potted plants from Sumatra and the Philippines in the 1920's and 1930's, and mycorrhizal infection came with them. When systematic introduction trials were started in the early 1950's some troubles appeared. Among other measures, a few seedlings of *Pinus caribaea* were sent to Australia to be infected and then returned to Malaya (Freezaillah, 1967), because importation of soil inoculum was prohibited. Griffiths (1965) also reported on failures in nurseries, which were overcome by inoculating the soil with surface litter of established pine stands. In general, however, Malaya nurseries are already well infected with mycorrhizal fungi and, therefore, trouble due to mycorrhizal deficiency is uncommon. Standard nursery operations do not include inoculation (Freezaillah, 1966a,b). The need for inoculation, however, is taken into consideration at the afforestation of grasslands and old agricultural soils (K. G. Singh, 1966).

The FAO manual for tropical Asia (Letourneux, 1957) also considers the necessity for mycorrhizal inoculation at the introduction of exotic pines, and detailed instructions for its performance are given. These are based mainly on the above-described experiences in the Philippines and Indonesia. Reference is also made to experiences in the former **Indochina** and in **Ceylon.**

There has been little practical experience of mycorrhizal inoculation in **India.** On the other hand, the success of pine and *Eucalyptus* plantations without artificial introduction of fungal symbionts is reported (Bakshi, 1966, 1967), and the manual of Indian silviculture (Champion and Trevor, 1938), for instance, does not include anything about inoculation of nurseries. The poor success of the first introduction of *Pinus caribaea* in 1937 (Mukherji and Thapar, 1961), as well as several failures of plantations may have been due to the original absence of mycorrhizal fungi, as has been pointed out by S. Singh and Kumar (1966).

There are only a few reports on the need for mycorrhizal inoculation when eucalypts are introduced as exotics into new areas. On the contrary, several authors have reported that inoculation of eucalypts is not necessary. Pryor (1956a,b) has pointed out, however, that the species of the group Renantherae have almost consistently failed, when tried outside their natural range, and suggested lack of suitable symbiotic fungi as the cause of the failure. Experiments with inoculation, conducted in **Iraq,** confirmed this hypothesis, although Levisohn (1958a) came to the opposite conclusion. This experience, however, has not been applied on a practical scale.

Soil inoculation with mycorrhizal fungi is not practiced in **Israel.** There are indigenous pine forests in the country, as well as forests of some other ectotrophic trees (*Quercus*), and, consequently, sufficient populations of mycorrhizal fungi are probably present in nursery soils. Furthermore, natural forests constitute a good source of airborne spores. Nursery-grown seedlings usually have mycorrhizae already at an early stage of development. Absence or poor development of mycorrhizae has sometimes been observed to be closely correlated with chlorosis and poor growth of the seedlings (Wahl and Reichert, 1953). Then, however, the reason is not the absence of appropriate fungal species, but unfavorable soil conditions. Both the growth of seedlings and mycorrhizal development can be corrected by altering soil conditions, such as drainage and aeration, for instance (Karschon, 1961).

According to oral information (Baltaxe, 1967), difficulties were encountered in early attempts to grow pines and cedars in some new nurseries in **Cyprus,** but they were overcome by soil inoculation.

D. Europe and the Soviet Union

The European literature includes a great number of experimental studies in which nonmycorrhizal seedlings and inoculated mycorrhizal ones have been grown side by side under similar conditions. Although the method of inoculation or other details of the experimental technique are often open to criticism, the results have almost consistently proved the great superiority of mycorrhizal seedlings and the beneficial influence of inoculation. Most of these studies, however, have not aimed at or resulted in practical applications in the afforestation of treeless areas, and therefore there is no point in trying to list them all. (For a more detailed review, see Levisohn, 1958b.) A few of many possible examples are reviewed from the practical point of view below.

Europe belongs to the temperate and cool climatic zones, wherein forests of ectotrophically mycorrhizal trees form the natural climax vegetation. Under such conditions practically all natural soils contain mycorrhizal fungi, and one may expect soil inoculation to have little or no effect. There are certain areas, however, which are naturally treeless, such as the steppes of southern Russia, or areas where forests have been destroyed hundreds or even thousands of years ago, as in many parts of the Mediterranean countries, resulting perhaps in the disappearance of mycorrhizal fungi from the soil. At the present time afforestation is also commonly conducted on old agricultural lands, which have been under cultivation for centuries, and then the question may arise as to whether it is necessary to restore the mycorrhiza-fungal population.

In Mediterranean Europe mycorrhizal inoculation does not seem necessary at the afforestation of treeless areas. In this respect conditions are similar to those of North Africa and Israel (cf. above). In **Spain,** for instance, in recent years some 100,000 hectares have been planted annually, and inoculation has never been practiced. Likewise in **Italy,** nurseries are usually located on agricultural land, but seedlings nonetheless develop mycorrhizae without any inoculation; these seedlings, when planted out into waste lands, such as denuded mountain slopes or abandoned fields, have generally grown successfully. Recent studies have shown, however, that both the survival and initial growth of seedlings can be considerably improved by inoculation, especially when pines are grown far from natural pine forests or older plantations. This was demonstrated, for instance, by a field experiment conducted in Sardinia on an area where pines had never grown before (Eccher and Rambelli, 1966). Uninoculated seedlings were mycorrhizal, indicating

that some local fungi were capable of forming mycorrhizae with *Pinus radiata;* seedlings that had been inoculated with soil from an old pine plantation grew much faster, however, and had mycorrhizae of a different type. Sporophores of *Boletus granulatus* also appeared around the inoculated seedlings, whereas they were absent in the uninoculated plots. This experiment and other corresponding experiences indicate that soil inoculation, i.e., the introduction of new and more efficient species of fungi, can be feasible and beneficial even if the soil already contains some other mycorrhiza-forming fungi. Heavy inoculation has proved particularly beneficial in connection with plantations on old agricultural soils where, because of a high pH or for other reasons, conditions are unfavorable for mycorrhizal fungi (Fassi, 1967; Rambelli, 1967). Therefore, at the present time inoculation is sometimes practiced at the afforestation of abandoned fields in Italy.

Inoculation is also practiced in Italian nurseries when pines are raised in steam-sterilized soil in polythene bags (Rambelli, 1967).

In **Austria** mycorrhizal inoculation has reached the stage of practical application in the restoration of forest to subalpine areas at and above the present timberline. As Moser (1956, 1958a,b, 1962, 1963) and Göbl (1962, 1965a) have pointed out, the most-efficient mycorrhizal symbionts of subalpine *Pinus cembra* forests, such as *Boletus* (*Suillus*) *plorans*, are lacking in the nurseries, which are usually located on agricultural soils in the valleys, and therefore a technique was developed to inoculate nursery soil with fungi of high altitudes (Moser, 1959). The technique has proved successful and is now generally practiced at *Pinus cembra* afforestation (Göbl, 1967). The Soil Biological Laboratory at Imst, Tirol, provides *Pinus cembra* nurseries with mycorrhizal inoculum, and inoculated seedlings have shown much better survival and initial growth after planting than seedlings with natural infection from the nursery soil (Moser, 1963). Experiments are also in progress to develop practical methods of mycorrhizal inoculation for spruce afforestations at high altitudes (Göbl, 1967).

In some other European countries interest in mycorrhizal inoculation has been concentrated around the problem of afforestation of former agricultural lands. Dominik (1958, 1961a,b), in **Poland,** noticed that although mycorrhiza-forming fungi are present in agricultural soils, they represent inferior types and the population density may be low. Therefore, direct seeding of pine usually meets with poor success on agricultural soils. Inoculation of agricultural soils with the natural fungal population of pine forests proved possible. Instead of large-scale soil

inoculation, however, Dominik recommended planting of mycorrhizal seedlings raised in a forest-soil nursery. When mycorrhizal seedlings with a good type of mycorrhizae are transplanted from a forest-soil nursery into former agricultural soil, they grow well. As the best method Dominik (1961b) recommended the use of mycorrhizal seedlings from forest-soil nurseries and, in addition, the application of a handful of forest soil in each planting hole, thus ensuring the introduction of both mycorrhizal and other indigenous fungi of pine forests and at the same time a natural environment for the mycorrhizal roots immediately after planting.

Similar results have been obtained in **Czechoslovakia.** Soil inoculation was not found necessary for the successful afforestation of old agricultural lands, although in some experiments inoculation improved survival, growth, and mycorrhiza formation in both field sowings and nurseries (Sobotka, 1957). Improvement has not been consistent, however, and these studies have not led to the practical application of inoculation in forestry. Instead, Sobotka (1963) has pointed out that mycorrhiza formation can be stimulated more effectively by changing the physicochemical conditions of the soil than by direct introduction of mycorrhizal fungi.

The favorable effect of forest humus on the growth of seedlings in nurseries has been observed in several countries (e.g., Rubtov, 1964), and forest litter or humus is generally applied to nursery compost or directly to nursery beds. It is not always clear, however, whether the beneficial effect is due to introduction of mycorrhizal fungi or other forest soil microbes, to favorable changes of pH or other soil properties, or to a combination of all these.

The famous experiments of Rayner (1934, 1936, 1939) and Rayner and Levisohn (1941) in England should also be mentioned in this connection. Although they did not lead to immediate practical application of soil inoculation in British forestry, they have greatly stimulated practically oriented research on mycorrhizae and nursery problems in general.

In the **Soviet Union**[2] most research on mycorrhizal inoculation, and practical application of it, have been connected with the establishment of oak shelterbelts in the steppes. Vyssotsky (1902) was the first to suggest that grassland soils might be deficient in the necessary mycorrhizal fungi and he later (1929) proposed a method for their artificial introduction. Some later papers (Pyatnitsky, 1932; Stepanov, 1932; Baraney,

[2] References to the Russian literature are mainly based on the textbook of Lobanow (1960) and articles by Lobanow (1963), Michowitsch (1963), and Puschkinskaja and Mischustin (1963).

1939, 1940) indicate that mycorrhizal inoculation was already practiced before World War II on an experimental and even on a practical scale in the establishment of oak shelterbelts.

When steppe afforestation was started on a large scale after World War II, an extensive research program was organized, in which several universities and other scientific institutes participated. This program covered all the problems important for the successful afforestation of treeless grassland, such as the presence or absence of mycorrhizal fungi in different areas and soils, the necessity and effect of inoculation, techniques of inoculation, and methods of stimulation of mycorrhiza formation. The main emphasis was placed on afforestation by direct seeding with oak and pine. Research projects were conducted in the years 1946 to 1953 and the results published in the proceedings of a Conference on the Mycotrophy of Plants (see Imshenetskii, 1967) and in several scientific journals of the Soviet Union. At the same time, in 1949 to 1952, the problem of steppe afforestation as a whole was studied by a joint expedition of experts of various related sciences (Sonn, 1955).

All these studies have been thoroughly reviewed by Lobanow (1960, 1963). The results are briefly summarized as follows: No complete failure of afforestations due to absence of mycorrhizal fungi was reported. Several authors, however, obtained such an improvement of survival and growth of seedlings that they considered inoculation justified or even necessary for the success of steppe afforestation; whereas in the opinion of some other authors the same effect can be obtained with simpler and less expensive methods, and the possible benefit to be derived from soil inoculation does not justify the trouble and expense involved. Sonn (1955) drew the conclusion that "the general opinion, that the steppe soils contain no mycorrhizal fungi, is not correct. There are fungi in grassland soils, which are able to form mycorrhizae with oak and other tree species, although their density may be there much lower than in forest soils. Therefore, artificial infection of oak with mycorrhizal fungi accelerates the formation of mycorrhizae in steppe soils and improves the growth of young oak seedlings." Lobanow (1960, p. 279), in turn, came to the empirical conclusion that artificial inoculation is advisable, although not necessary, in chernozem soils, but absolutely necessary in chestnut soils. This recommendation has probably been acted upon in the later afforestation of the steppes.

More recently the absence of mycorrhizal fungi and the necessity of inoculation have also been reported from the Central Asian part of the Soviet Union (Slavkina, 1963).

E. North America

The need for inoculation became obvious when shelterbelt plantations of conifers were started in the early 1930's in the prairies of the central **United States.**

McComb (1938) was the first to report on failures of nurseries in treeless areas and the sudden recovery of seedlings as a result of inoculation. Probably trouble had been experienced even earlier in growing pines in the prairies, since systematic experiments with mycorrhizal inoculation had been started in 1933 in four nurseries of North Dakota (Stoeckeler and Slabauch, 1965). The experiments of Hatch (1936, 1937), D. P. White (1941), and others revealed the absence of suitable mycorrhizal fungi in grassland soils. Mycorrhizal problems in relation to afforestation of prairies have been intensively studied at the University of Wisconsin; these studies have been summarized by Wilde (1954).

In the United States the need for mycorrhizal inoculation is restricted to nurseries. Direct seeding is not practiced in the afforestation of prairies and other treeless areas, and when mycorrhizal nursery-grown seedlings have been transplanted into prairie soil, their growth and mycorrhizal development has always continued without difficulty. The present American practice is probably best expressed in the handbook of Stoeckeler and Slabauch (1965), which recommends soil inoculation for new nurseries in the prairies. Inoculation alone, however, is not always sufficient, but a change of some soil properties, such as acidification, for instance, is often necessary to favor the mycorrhizal fungi. The possible need of mycorrhizal inoculation in new nurseries in some other areas, which have been treeless for a long time, is also considered (Stoeckeler and Jones, 1957).

F. Central and South America

The history of mycorrhizal introduction is perhaps better documented in **Puerto Rico** than anywhere else (Briscoe, 1959; Hacskaylo and Vozzo, 1967; Vozzo, 1967). Unsuccessful attempts to introduce pines to the island were made repeatedly for almost 30 years, since 1928. Altogether, 27 species of *Pinus* were tested. The seed usually germinated well and the seedlings grew rapidly for a few months, but then turned yellow and gradually died, either in the nursery or soon after they were planted out.

In July of 1955, soil was brought by Huckenpahler (1967) in eight cardboard boxes from a *Pinus taeda* and a *P. echinata* plantation in

North Carolina and was immediately applied to 32 one-year-old *P. elliottii* seedlings, which had been planted out near the town of Maricao in the western mountains, another 32 seedlings serving as controls. The inoculum was worked into the soil around the seedlings, and to ensure similar conditions for all the seedlings, the soil around the control seedlings was cultivated in the same way but without inoculation. When Hacskaylo (1959) visited the area in 1959, some of the control seedlings were still alive, 10 to 18 inches high and yellow, whereas the inoculated trees had dark green foliage and were 2 to 6 feet high. In 1967, the remaining inoculated trees were up to 10 m tall and 4 to 5 inches thick at breast height, whereas nothing was left of the controls. Soil from this first successful pine plantation was used between 1956 and 1959 to inoculate nurseries and recently planted seedlings in other parts of the island.

The second importation took place in 1958. One-year-old mycorrhizal seedlings of *Pinus elliottii* were brought from Florida and similar seedlings of *P. taeda* from Mississippi. They were planted out in seven localities and have grown satisfactorily. A few of the same seedlings were planted in a pine seedling bed at Catalina nursery, and mycorrhizal infection spread from them to adjacent seedlings, as was clearly indicated by the sudden change of color and improved vigor of the seedlings (Hacskaylo, 1959). The soil of this bed was then used in subsequent years as inoculum in the nursery.

Mycorrhizal inoculum was imported for the third time in 1959. Hacskaylo of the Forest Physiology Laboratory of the U. S. Forest Service sent duff and humus of a natural *Pinus virginiana* stand from Beltsville, Maryland, to the Institute of Tropical Forestry, and a seedbed of *P. caribaea* was successfully inoculated with this "Maryland mixture." The same inoculum has since then been propagated and continuously used at the nursery of the Institute.

After the introduction of mycorrhizal inocula there has been no difficulty in growing pines in Puerto Rico. Inoculation is now one of the standard nursery operations (Marrero, 1962). As reported above, there have been three separate importations, and today it is impossible to be sure of the origin of the infection at different plantations.

Mycorrhizal inoculum was brought to Trinidad in 1946. Attempts at growing pines before that year had all failed, probably owing to lack of mycorrhizal fungi (Anonymous, 1947b). After visiting the island in 1946, Lamb (1956, 1967) sent *Pinus caribaea* seed and four gallons of topsoil of a natural pine forest from British Honduras. Seed was sown

in a nursery and the soil was inoculated with the imported soil. From these seedlings originated the first successful pine plantation of Trinidad, at Arena. The present two pine nurseries in Trinidad (Cumuto and Cap-de-Ville) have been inoculated with soil from this plantation. Today mycorrhizal inoculation is regularly practiced in both nurseries. Seedlings are raised in wooden boxes, and pine soil is always added. Probably there has been no other importation of mycorrhizal inoculum to Trinidad.

Pinus massoniana was introduced to **Jamaica,** probably as potted seedlings, before 1900 and planted at Cinchona Botanical Garden, where the trees are still growing and regenerating naturally (Lamb, 1968). All the other attempts to grow pines in Jamaica failed until about 1950, when soil inoculum was brought from British Honduras by some foresters carrying small tin boxes in their pockets (Swabey, 1967). The soil was first used to inoculate a few pots with pine seedlings, then the soil of these pots was applied to a nursery bed, and later other nurseries were inoculated with soil from this first nursery.

Mycorrhizal soil has also been brought from British Honduras to **Surinam,** and only after inoculation have pines grown successfully (van Suchtelen, 1962).

The first inoculum to **Costa Rica** probably came in 1960 with potted pine seedlings taken from natural pine stands of Honduras (Vega Condori, 1962). Inoculation with both mycorrhizal roots and soil has proved successful, whereas uninoculated seedlings have failed (Vega Condori, 1964). Pure cultures of mycorrhizal fungi have also been imported and inoculation has been successful (Volkart, 1964).

Exotic pines are grown extensively in the State of São Paulo in **Brazil.** Large-scale plantations were started about 10 years ago but some pine trees had been planted long before as park trees and ornamentals. There are no records of the importation of mycorrhizal inoculum. Inoculation is generally practiced, however. In the nursery of the Forest Research Institute (Horto Florestal) at São Paulo, pine-forest soil is regularly added to the potting mixture for pine seedlings, and pine-needle litter is also used as mulch. When new nurseries have been started during the last 10 years, the first crop has usually been transplants brought from Horto Florestal nursery, and thus the soil of new nurseries has become infected. Bags of pine soil, mixed with sporophores of mycorrhizal fungi, have also been sent on request to other nurseries from a pine plantation at Horto Florestal for inoculation purposes (Cardoso May, 1967).

In **Argentina** there are no conclusive records of failure of plantations due to mycorrhizal deficiency. Inoculation has proved beneficial and

practicable, however, and is even considered necessary when afforestation is started on new areas far from existing plantations. A special inoculation technique has been developed (Takacs, 1961a,b, 1967a). The mycorrhiza laboratory at Castelar (Instituto Nacional de Technologia y Agropecuaria) provides new nurseries with mycorrhizal inoculum, which is used for the first inoculation, and if a nursery is extended, soil from the old beds is used to inoculate the new section (Takacs, 1967b).

As far as is known, no mycorrhizal inoculation has ever been practiced in **Chile**, in spite of the fact that exotic conifers are grown extensively.

III. Technique of Inoculation

A. Soil Inoculum

Soil of natural forests, plantations or nurseries has been the form of mycorrhizal inoculum most widely used. Mycorrhizal soil has been imported, for instance, into the East African countries, Zambia, Malawi, Swaziland, Nigeria, Congo, New Guinea, Jamaica, Trinidad, and Puerto Rico. Furthermore, soil inocula have sometimes been transported long distance within countries.

The techniques of applying soil inoculum vary according to the nursery practice. If the seedlings are raised in beds of natural soil, as in Australia, the first inoculation is usually made by spreading a thin layer of mycorrhizal soil, 1 to 2 cm thick, on top of the bed and mixing it with the surface soil. No later inoculation is usually needed. Such a method is also recommended by Letourneux (1957).

If the seedlings are raised on artificial soil in pots, polythene tubes, trays, or Swaziland beds, as is done in most tropical and subtropical countries, the pine soil inoculum is usually incorporated into the soil mixture used for potting or transplant beds. Thus, the mixture of May (1953), which was generally recommended for East African nurseries, contained 10 to 20% of pine soil. Today African nurseries usually use simpler transplanting mixtures, consisting of natural forest topsoil with or without addition of sand or manure (Procter, 1967). The proportion of pine soil—if it is used at all—varies from 10 to 50% in different nurseries. If Swaziland beds are used, 30 to 50% of the soil remains in the beds after the lifting of the seedlings and can be mixed with new soil to serve as inoculum; no new pine soil is then required. Some African nurseries, however, discharge all the old soil for fear of disease and prefer to make a new mixture with fresh pine soil every year.

The amount of pine soil was a problem in former times, when it was not readily available. Parry (1953) refers to the great difficulty of obtaining sufficient amounts of mycorrhizal soil when afforestation projects are started in new areas. Under such conditions it is advisable to use as small an amount as is still effective; 10% has been considered sufficient for pots and Swaziland beds. On the other hand, if the nursery is surrounded by pine plantations, there is no need for saving. Topsoil from pine plantations can be used as such for potting, sometimes with the addition of manure or commercial fertilizer.

Soil inoculum is usually added to the transplanting mixture. Some nurseries use the same soil mixture for both seedbeds and transplant beds, whereas most nurseries use sand or a mixture of sand and soil for seed germination, without adding mycorrhizal inoculum. Seedbed inoculation was formerly practiced, at least in Rhodesia (Southern Rhodesia Forestry Commission, 1959) and Uganda (Stuart-Smith, 1953). In early experiments in Nyasaland, both seedbeds and transplant beds were inoculated (Clements, 1941). Inoculation of transplant beds alone, however, may be sufficient and preferable.

In the tropical nursery technique, seedlings are transplanted soon after germination, before they have any laterals or short roots, and inoculation of seedbeds would therefore be useless. Recently, however, some nurseries have eliminated the transplanting of seedlings, and seed is sown directly into inoculated soil in polythene tubes. In Southern Queensland, Australia, *Pinus elliottii* and *P. radiata* are raised in beds without transplanting, whereas *P. caribaea* is transplanted into polythene tubes. Inoculation of the tubing soil is then not necessary, because the seedlings carry the infection from the seedbed.

Instead of forest topsoil, pine-forest litter is used in some nurseries. Thus, at Olmotonyi nursery in Tanzania the potting mixture contained 20 to 30% by volume of pine-needle litter. The primary purpose of this measure was to increase the organic matter content and acidity of the substrate, because both the soil and irrigation water were slightly alkaline, but at the same time mycorrhizal infection was secured. Pine-needle litter is also used as mulch in nurseries in the southeastern United States and in Brazil, and has proved to be an efficient carrier of mycorrhizal infection. If the nursery soil has been sterilized with steam or methyl bromide, as is customary in the United States, unsterilized pine-needle mulch greatly promotes the formation of mycorrhizae.

I saw a special kind of inoculation technique at Horto Florestal nursery in São Paulo, Brazil. Seedlings are raised in blocks of compressed

soil with a central hole. The material for the blocks is a mixture of soil, clay, and bagasse, while the central hole, where the seed is sown or small seedlings transplanted, is filled with pine-forest soil. In addition to that, pine-needle litter is used as mulch.

Instead of incorporating soil inoculum into the potting mixture, polythene tubes or bags are inoculated individually in some nurseries, either at the time of transplanting or a few weeks later. In Nigerian nurseries, seedlings that have been transplanted into polythene bags are inoculated about 1 month after transplantation with one teaspoonful of pine soil, which is put in a small hole near the base of the seedling (Fig. 2). A

FIG. 2. Inoculation of individual seedlings of *Pinus caribaea* in polythene tubes. (Bukuru nursery, Nigeria; photograph taken October 23, 1967.)

similar method is used in Puerto Rico. Thus the inoculation is performed at the time when the seedlings already have short roots, which can be infected immediately.

In some Italian nurseries a special machine is used for filling the polythene bags, and the inoculum is added to the bags manually. The inoculum cannot be mixed with the soil before filling for two reasons: (1) the soil is sterilized and the inoculum must be added after sterilization, and (2) the soil has to be dry to go through the machine, whereas the inoculum must be kept moist (Rambelli, 1967).

Mycorrhizal soil or forest litter has also been used in the Soviet Union to inoculate pine seed and acorns when shelterbelts have been established in the steppes by the direct seeding method (Lobanow, 1960). According to Krasovskaya and Smirnova (1959), soaking of acorns in a water suspension of forest humus is sufficient for mycorrhization and is recommended in cases where large amounts of mycorrhizal soil and organic matter are difficult to supply. The best infection and development of seedlings, however, is obtained if both mycorrhizal soil and organic manure are applied to the sowing patches. Lobanow (1960, pp. 280–284) gives detailed instructions for the preparation, storage, and application of mycorrhizal soil.

Inoculation of pine seed with mycorrhizal soil has also been tried in Australia (Forrest, 1966). Broadcasting the seed and mycorrhizal soil simultaneously has been successful in small scale trials. The need for large amounts of soil inoculum (about 1 ton per acre), however, makes this method impracticable on a large scale. Experiments with seed pelleting, i.e., coating pine seed with a mycorrhizal soil slurry with a suitable adhesive, have not yet resulted in practicable application.

When mycorrhizal soil inoculum is shipped for long distances, it should be kept moist and transportation time should be minimal. There are only a few records of the drying of the inoculum and death of the fungi during transportation. Usually transportation has not taken more than 2 weeks. Probably failures have not been uncommon, however, since, according to Letourneux (1957), "nine times out of ten failure occurs if the shipment takes more than eight days."

It is not known exactly how long mycorrhizal fungi can remain viable in soil inoculum under various storage conditions. According to Olatoye (1966), inoculum could be stored for 15 months without deterioration when kept moist in polythene bags. Likewise, Lobanow (1960) and his associates have reported that mycorrhizal soil collected in the autumn and kept through the winter in moist condition is as effective as freshly collected soil. Other experiments have shown that some mycorrhizal fungi can survive, probably in the form of spores or sclerotia, for a long time even in dry soil. Storage of soil in a dry condition may, however, exert a considerable selective influence on its fungal population, some species being more drought-resistant than others. The black mycorrhizal fungus *Cenococcum graniforme* is particularly drought-resistant (Worley and Hacskaylo, 1959; Trappe, 1962b). In some experiments of Hatch (1937) and Mikola (1948), inoculation with dry forest humus resulted in black mycorrhizae only, whereas inoculation with the same soil when

still moist mainly produced light-colored mycorrhizae. Thus, there is always a risk that the most beneficial species will die out, although some other mycorrhiza-formers may still survive after prolonged storage even in a dry condition. Lobanow (1960), however, recommends storage of inoculum over the winter in a dry condition.

In African nurseries mycorrhizal soil is usually stored under a shelter, or other measures are taken to protect it from direct sunshine and drying. It may happen that if a heap of pine soil or tubing mixture is exposed to direct sun, the surface layer becomes very warm and dry, whereas the interior still remains sufficiently moist. Owing to the death of fungi in the dried and overheated surface layer, the use of such a soil for tubing may result in uneven survival and growth of the tubed seedlings, because of lack of infection in some tubes (Foot, 1967). Inadequate mixing of the inoculum with the tubing soil may produce the same result.

Soil as a mycorrhizal inoculum has both advantages and disadvantages. The main advantages are the great ease and reliability with which inoculation can be done. It may be difficult to keep the fungi in viable condition during long distance transportation, but when the inoculum has once been introduced, it can be rapidly propagated and distributed. If the nursery is located close to existing plantations, large amounts of inoculum can be used, which guarantees rapid and even infection of all the seedlings.

If the inoculum comes from natural stands or healthy and thriving plantations, it presumably contains a balanced population of different species of mycorrhizal fungi—provided, of course, that no species have died during transportation. Mycorrhizal nursery soil, in turn, is a very homogeneous material for inoculating new beds or even new nurseries, and thus uniform distribution of mycorrhizal fungi can be achieved from the very beginning.

The large volume and great weight of soil needed for inoculation is perhaps the greatest technical drawback of the method. Lobanow (1960), for instance, recommended that for direct seeding of acorns 100 g of inoculum should be added to each sowing hole, corresponding to 2 m^3 per hectare. Procurement and transportation of such amounts of mycorrhizal soil to afforestation areas may be impractical or impossible.

There are also biological reasons why the use of soil inoculum has been strongly criticized by many scientists (e.g., Harley, 1959). Soil inoculum contains an indiscriminate mixture of all the fungal species and other soil organisms of the site from which it is taken. From the theoretical point of view it would be desirable that the most efficient

mycorrhizal fungi should be favored and the less efficient ones eliminated. The greatest danger, however, is that soil inoculum may also contain germs of parasites and diseases. Practical instructions (Lobanow, 1960) advise that soil inoculum must not be taken from stands where *Armillaria* is present, or from nurseries infested with damping-off. Such precautions, however, cannot eliminate the risk of introduction of diseases. Soil inoculum cannot be disinfected without also killing the mycorrhizal fungi. The danger of introducing diseases is rather slight if soil is brought from healthy natural stands to nurseries of the same area. Considerably more risk is involved in carrying soil from one nursery to another, when damping-off fungi, nematodes, and other nursery pests may be introduced at the same time. Importation of soil from other countries always involves a risk of introduction of new diseases.

For the above reason there has been great difficulty in bringing mycorrhizal inoculum to many countries. To protect the country from plant and animal diseases, most countries have strict quarantine regulations, prohibiting or restricting the importation of unsterilized soil and fresh plant material. In fact, the first introduction of mycorrhizal inocula to several areas has taken place illegally. This may be one reason why the introduction of mycorrhizal inoculation to many countries is very poorly recorded. Probably there have been several cases of smuggling that were never documented. The troubles experienced in getting mycorrhizal soil to Zambia have already been described. Fortunately, no cases are known where plant diseases have been introduced into a country in a shipment of mycorrhizal inoculum, but the risk still exists, nonetheless.

The use of soil as mycorrhizal inoculum is not a satisfactory method for scientific experiments either, because the establishment of exactly comparable control treatments is difficult. Likewise, it is hard to judge with certainty whether the possible response to inoculation is due to mycorrhizal fungi only or to some other factors present in the soil used as inoculum.

B. Mycorrhizal Seedlings

Mycorrhizal seedlings, which are transplanted from the nursery into the field, carry mycorrhizal infection in their roots and no new inoculation in the field is usually needed. Grassland soils, which may be naturally devoid of mycorrhizal fungi, apparently do not contain any antagonistic factors preventing the further development of mycorrhizae (D. P. White, 1941; Wilde, 1954).

Mycorrhizal seedlings were first used for inoculation of new nursery beds in Indonesia (Rocloffs, 1930). Since then the same technique has been regularly used there and is still used today (Becking, 1950; van Alphen de Veer *et al.*, 1954; Weck, 1961; Lamb, 1966; Cooling, 1967a). Therefore this method can be called "Indonesian" although the technique was known quite early in Australia as well (Kessell, 1927).

According to the Indonesian method, mycorrhizal seedlings or "mother trees" are planted at 1- to 2-m intervals in the nursery beds. The following year, young seedlings, 6 to 8 weeks old, are planted around these mother trees at 10 by 10-cm spacing. A healthy green color and start of growth are visible indications of the spread of the infection from the mother trees to the surrounding transplants. According to Becking (1950) and van Alphen de Veer *et al.* (1954), the beds become sufficiently infected in two years, after which time mother trees are no longer necessary. Cooling (1967a), however, was surprised that mother trees were still used in a nursery about 20 years old and located in the midst of a thriving pine plantation.

Inoculation can also be performed in such a way that when the seedlings are lifted, a few seedlings are left growing in the beds to inoculate the next crop.

The use of mother trees is said to be the only possible way to inoculate *Pinus merkusii* seedlings in the nursery. Trials of mycorrhizal soil from pine plantations have failed (van Alphen de Veer *et al.*, 1954). In Zambia, however, *Pinus merkusii* is raised successfully in nurseries without mother trees (Holmes, 1968).

There are relatively few other areas where mycorrhizal seedlings are used for nursery inoculation. I have seen such a practice on a small scale in Nigeria and New Guinea. At the Federal Forest Research Institute in Ibadan, Nigeria, this method is used for the propagation of mycorrhizal inoculum. Mycorrhizal seedlings are planted in the middle of nursery beds at 1-m intervals, and around them young seedlings are planted. After the young seedlings have developed mycorrhizae, they are potted and sent to other nurseries, to be used there as mother trees. The spread of the infection in the Ibadan nursery is slow, only 40 to 50 cm a year. In sterilized soil the infection spreads considerably faster. Too high a pH (about 7) is suspected to be the reason for the slow advance of mycorrhizal infection (Lamb, 1967; Lowe, 1967).

Mycorrhizal seedlings are also used for seedbed inoculation in the Congo, whereas pine soil is added to the transplanting substrate (Groulez, 1967).

Where the Indonesian technique of inoculation is practiced, the seedlings must be raised in beds. Polythene tubes or bags, however, are currently preferred to open beds in tropical nurseries. According to practical experience, the tubing technique can be adopted after two or three years' use of the Indonesian method, when the soil of the beds is already sufficiently infected to be applied to the tubing mixture.

The Indonesian technique is also used for "bulleted" pine plants in Madagascar (see figure in Bégué, 1964). According to Poupon (1967) the method is successful, whereas failure has resulted from inoculation with mycorrhizal soil.

As was described in Section II, the first introduction of mycorrhizal infection to some countries took place in the roots of living seedlings. Thus, mycorrhizal pine seedlings were brought to Fiji, Malaya, and the Northern Territory of Australia. One shipment of mycorrhizal seedlings was also brought to Puerto Rico. In fact, probably in most countries mycorrhizal infection first arrived with imported seedlings of exotic trees, long before anything was known about mycorrhizae at all. Such early unintentional introductions will be discussed later (Section IV, B).

Living seedlings can also be used for preservation of mycorrhizal infection. Such an example was described from Zambia by Cooling (1967): When Sanfya nursery near Lake Bangweolo was established in 1955, several bags of mycorrhizal soil were brought from the old Dola Hill nursery. It was mixed with the local soil to make an oblong pile, which was covered with pine-needle litter. A row of pine seedlings was then planted on the top of the pile. Both the needle litter and the first pine seedlings were also brought from Dola Hill. When the soil of the pile was used to fill polythene tubes, a new soil pile with new pine seedlings was built up. This was a safety measure to preserve the mycorrhizal inoculum in active condition over the dry season, because in Zambia seedlings are kept in the nursery for 7 to 9 months only, and thus there is a period of 3 to 5 months every year between the lifting of the seedlings and a new sowing.

The use of mycorrhizal seedlings seems to be a very reliable method of inoculation. The difficulties of transporting large volumes of soil inoculum for long distances are avoided by using mycorrhizal plants. On the other hand, living seedlings need more care than a parcel of soil during transportation.

With respect to the risk of introduction of diseases, mycorrhizal seedlings may be safer than soil inocula. The health of potted seedlings can be better controlled than large amounts of unsterilized soil. Special

measures can be taken to minimize the risk. It is possible, for instance, to wash the roots and then transplant the seedlings into sterilized soil. Particular care is necessary if living seedlings are transported from one country to another. Forest diseases have reached new countries with shipments of living plants, although probably this has not happened in those relatively few cases in which seedlings have been imported particularly for mycorrhizal inoculation.

Mycorrhizal roots are often used to infect seedlings in scientific experiments (e.g., Stone, 1950) and are preferred to soil inoculum.

C. Pure Cultures

Theoretically, the use of pure cultures of mycorrhizal fungi would be by far the best method of inoculation. With pure-culture inoculation, the species of fungi could be chosen and all risk of introducing diseases would be eliminated. For successful use of pure cultures, however, we should know which fungal species are the most beneficial symbionts and which are less efficient, how to culture the superior species to produce sufficient amounts of inoculum, and how to perform the inoculation in practical nursery or field conditions. In all these respects our knowledge is still very deficient, and therefore pure-culture inoculation has been little practiced in forestry. On the other hand, pure cultures have been extensively used in scientific experiments, often aiming at practical applications as well.

The first difficulty is to obtain pure cultures. The techniques of isolation and cultivation of soil fungi, as developed by Melin (1936) and others, are known and have been extensively used in mycological studies. It must be pointed out, however, that mycorrhizal fungi are in general difficult to isolate and that most of them grow very slowly in pure culture. There are a great number of forest soil fungi, probably mycorrhizal, which have never been isolated. Thus, it is possible that the most obligate symbionts—and perhaps the most beneficial ones too—have never been isolated or grown in pure culture. This applies, for instance, to numerous species of *Cortinarius*, *Russula*, and *Lactarius*. Fortunately, several species of *Boletus*, which are some of the commonest and probably also most efficient mycorrhizal fungi of pine forests, grow reasonably well in pure culture. Therefore *Boletus* species have mostly been used in pure-culture experiments, and attempts at practical field inoculation have usually been made with them.

Data on the application of the pure-culture technique on a field scale are available only from Austria and Argentina.

The Austrian technique is mainly based on the extensive studies of Moser (1958b,c, 1959). The typical and probably most active mycorrhizal fungus of subalpine *Pinus cembra* forests, *Boletus plorans*, which forms tuberous mycorrhizae with a white mantle, is apparently absent in the nursery soils of the valleys and also in the alpine meadows, which were deforested centuries ago and should now be replanted (Göbl, 1965a). The technique, therefore, aims at inoculation of *Pinus cembra* seedlings with *Boletus plorans* in the nursery. Since nursery soils may contain some other mycorrhiza-forming fungi, a heavy application of inoculum is necessary and a large amount of mycelium is needed. On the basis of the prolonged experiments of Moser (1958c) a routine technique has been developed, which is now used at the Soil Biological Institute in Imst (Göbl, 1967).

Boletus plorans is first grown on Moser's (1958b, p. 36) nutrient solution in Erlenmeyer flasks. From the flasks the mycelia are transferred to the same solution in 10-liter tanks, which are aerated for 2 or 3 hours daily. After 3 or 4 months the liquid with mycelia is poured into 5-liter flasks with a sterilized mixture of vermiculite and shredded peat, and a sufficient amount of the same nutrient solution is added to moisten the substrate. Proper moisture and aeration are important for the growth of the fungus. *Boletus plorans* will grow throughout the substrate in a few months, after which the inoculum is ready for use.

Moser (1959) also tried different times of inoculation. It is important that the fungus come into contact with actively growing roots as soon as possible after inoculation; therefore inoculation at the time of transplanting has proved most successful. In Austrian nursery practice *Pinus cembra* is transplanted at the cotyledon stage, when the first lateral roots are emerging. Transplanting and inoculation can be done at any time of the summer; late inoculation, however, has been less successful, probably because the low soil temperature in the autumn prevents the growth of the fungus.

The inoculum is sent from the laboratory to the nursery in polythene bags not more than three days before transplanting. It is applied to the beds at a rate of 3 to 4 liters per square meter and worked lightly into the surface soil. Seedlings are immediately planted in the inoculated bed. The success of the inoculation also depends on the physical and chemical properties of the nursery soil. It is not enough merely to introduce the fungus into the soil, but the conditions must be suitable for its further growth. Good aeration, moderate acidity, high organic matter content, and application of phosphorus fertilizers promote the growth and infectivity of *Boletus plorans*.

Inoculation is repeated, if possible, at every transplanting, regardless of whether the soil has been inoculated before or not. Apparently conditions are not favorable for the subalpine *Boletus plorans* in the nursery soils of valley bottoms, and therefore the population must be strengthened from time to time. The greatest difficulty in the Austrian inoculation method is the very large amount of inoculum needed (3 liters per square meter corresponds to 30 m^3 per hectare). For the time being the Soil Biological Institute at Imst is not able to provide all *Pinus cembra* nurseries with pure-culture inoculum, but a great many *P. cembra* seedlings are produced without inoculation, or soil from natural stands is used as inoculum.

Pinus cembra is so far the only species for which pure-culture inoculation is practiced in Austria. Larch, for instance, does not need any inoculation, since apparently in all nurseries the seedlings become spontaneously infected with *Boletus elegans* (*Suillus grevillei*), which is probably the most suitable symbiont for larch. Experiments on pure-culture inoculation of spruce have been started at the Soil Biological Institute (Göbl, 1967), but have not yet reached the stage of practical application. Since it is not yet known which fungal species are the most efficient symbionts for spruce under local conditions, the mycorrhizal relations of subalpine spruce forest were first thoroughly studied (Göbl, 1965b) and a mixture of pure cultures of several species are used in the first inoculations.

In Argentina, the problem is rather different. Inoculation is practiced there at the establishment of new nurseries in formerly treeless areas, where mycorrhizal fungi are presumably lacking. The pure-culture technique has been introduced by Takacs (1961b, 1964, 1967a,b). The idea was taken from Hungarian studies (Bokor, 1954, 1958). The technique of preparation of the inoculum is rather similar to that of Moser. Fungi are first grown in liquid cultures (Hagem solution) and then transferred to 200-ml flasks containing a peat or wheat-chaff substrate moistened with the same nutrient solution. After two months incubation, the inoculum is ready for distribution to the nursery. The stock cultures are maintained and the inocula raised in the mycorrhiza laboratory at Castelar.

At least the following fungi are used for inoculation: *Amanita verna*, *Boletus auripolus*, *B. bovinus*, *B. granulatus*, *B. luteus*, *Hebeloma crustuliniforme*, *Russula alutacea*, *Scleroderma verrucosum*, and *S. vulgare*. Because it is not known which of these species is preferable under different conditions, a mixture of three or four species is used for inoculation. Five flasks of each of these species are sent from the laboratory to a new

nursery. On arrival at the nursery the contents of each flask are mixed with 5 to 10 kg of sterilized soil or litter, which is then kept moist for three weeks before application to the nursery beds. Twenty kilograms of such inoculated soil is sufficient to inoculate 80 to 100 m^2 of nursery beds. Thus, with 20 flasks of pure-culture inocula, some 100 kg of soil inoculum can be prepared, which is enough for a nursery area of 500 m^2. Inoculation is performed in connection with seedbed preparation, immediately before sowing. Ordinarily, the nursery is inoculated only once, before the first sowing. If difficulties arise, however, which are suspected to be due to defective mycorrhization, a pure-culture inoculum can also be applied later between the seedling rows.

Practical inoculation with pure cultures is still young in both Austria and Argentina, hardly having progressed beyond the experimental stage. So far, inoculation has been practiced on a relatively small scale, and published results of field applications are not yet available. More conclusive results may be expected in the near future.

Experiments in several other countries have also been aimed at practical field application of the pure-culture technique. In the Soviet Union considerable progress has been reported (Lobanow, 1960). Thus, a technique for mass production of pure-culture inocula is presumably available (Voznyakovskaya and Ryžkova, 1956). Gendina (1957) used pure culture of *Boletus luteus* to inoculate pine seed before sowing. Seed was first surface-sterilized and soaked in a water suspension of the fungal mycelia and then sown in the field or nursery. Experiments were conducted on a relatively large scale in ten different areas, and positive effects of the inoculation have been reported. Successful inoculation of oak seed with pure cultures has been reported by Fedotova (1956).

Lobanow (1960) lists several other authors who have reported good effects of pure-culture inoculation on oak seedlings; the technique of inoculation, however, is not described in detail. The Russian experiments have also been reviewed by Levisohn (1958b). Shemakhanova (1961) and Puschkinskaja and Mischustin (1963) have published results of inoculation of unsterilized soil with pure cultures of several fungi; pine was best promoted by *Boletus bovinus*, *B. luteus*, *Scleroderma verrucosum* and *Cenococcum graniforme*, whereas *Amanita muscaria*, *A. rubescens* and *Boletus subtomentosus* were most promising for oak.

Successful inoculation of unsterilized soil with pure cultures on an experimental scale have already been reported by Hatch (1936), Young (1940), Rayner and Levisohn (1941), and others. On the other hand, there are numerous instances of less satisfactory or inconsistent results

of pure-culture inoculation. In general, scientists are more inclined to publish positive results of their experiments than failures, and therefore failures are probably more frequent than would be concluded from the literature. In a few cases only, and then mainly in controlled experiments under aseptic conditions, has inoculation proved harmful. Sometimes the fungus has even overwhelmed and killed the seedling, i.e., a balanced symbiosis was not established. More often, under nonsterile field conditions in particular, inoculation has had no visible effect at all, probably owing to the early death of the inoculum; in other words, the introduced fungus was not able to compete with the indigenous microbial population of the soil.

Pure-culture inoculation is the only acceptable method for strictly scientific studies and is mainly used in experiments under aseptic conditions. The possibilities for practical field use, however, are still rather limited. There are both technical difficulties and too many unknown factors involved. The most important problems to be solved before successful application of the pure-culture technique are the elimination of competition from other fungi present and the selection of suitable fungal species for different tree species and ecological conditions.

For the elimination of competition, soil sterilization is probably the only practicable method. Introduction of new fungi into unsterilized soil is possibly only by using a large amount of inoculum and at the same time promoting the new fungus by acidification, addition of organic matter, or other changes of soil conditions. Inoculation of sterilized soil with some fungal species seems to be fairly easy (Mikola, 1965; Laiho, 1965, 1967), whereas some other species consistently fail. Soil sterilization is nowadays commonly used in forest nurseries to destroy damping-off and other pests, but it may also destroy mycorrhizal fungi of the soil and consequently make inoculation necessary. Such cases are known at least in New Zealand and the United States. Thus, soil sterilization increases both the importance of mycorrhizal inoculation and the possibilities of the use of pure cultures.

So far, the fungi preferred for inoculation are those which are easy to isolate and grow rapidly in pure culture. It is doubtful, however, whether the same species are always the most efficient symbionts of trees. Physiological and ecological experiments have revealed great differences between individual species. The early experiments of Rosendahl (1942) suggested that different mycorrhizal species differ in their ability to dissolve potassium and phosphorus from rock minerals. In respect to phosphorus this has recently been confirmed by Bowen and Theodorou

(1967). Differences in the ability to release nitrogen from raw humus have been shown by Lundeberg (1967).

The same fungi are not always the species best suited to different ecological conditions, as is clearly shown by the experiments of Levisohn (1957, 1960) and Shemakhanova (1961). In nature, each individual tree is usually associated with several species of fungi (Zak and Marx, 1964). It is possible that different fungi benefit the host tree in different ways, and therefore a single species may not provide as efficient an inoculum as several species together. Such a hypothesis is supported, for instance, by the inoculation experiments in Puerto Rico (Vozzo, 1967; Hacskaylo and Vozzo, 1967): inoculation with mycorrhizal soil proved more beneficial than any of the pure cultures. The same kind of result was obtained by Mikola (1967), forest humus being a more efficient inoculum than a pure culture.

Thus, we know very little about which fungal species should be preferred for pure-culture inoculation. Some indications, however, are already available. There is strong evidence, both empirical and experimental, for instance, that *Boletus granulatus*, probably the most widespread mycorrhizal fungus in exotic pine plantations around the world, is one of the best species. Apparently, *B. plorans* is very suitable for the particular local conditions of the Austrian mountains. At the present stage of our knowledge, the Argentinian method of using a mixture of pure cultures of several species is based on sound reasoning.

D. Spores and Fruiting Bodies

It is generally known that mycorrhizal fungi spread easily through spores (Robertson, 1954), even to such a degree that seedlings raised from surface-sterilized seed in autoclaved soil in open pots develop mycorrhizae due to outside infection. Quite naturally, then, people have also tried mycorrhizal inoculation by using spores or fruiting bodies of mycorrhizal fungi.

Large amounts of *Rhizopogon luteolus* sporophores were used to inoculate nursery soils in Western Australia in the 1920's (Kessell and Stoate, 1938), and collecting of fungal fruiting bodies and their application to nursery soil is said to be a common practice in the Philippines (Lamb, 1966). There are also several sporadic references to small-scale field experiments. The beneficial influence of inoculation of *Eucalyptus* with *Pisolithus tinctorius* sporophores has been reported from South Africa (Palmer and Pitman, 1961). Levisohn (1956) also used *R. luteolus* sporophores to inoculate nursery beds in some experiments.

In all the above examples the technique has been the same. Fresh sporophores are collected and crushed and mixed with the surface soil of nursery beds. Dried sporophores have also been tried, although the results of these trials have not been published. Thus, dried sporophores of *Boletus luteus* were used in 1958 in two nurseries near the Kenyan east coast; the effect was probably positive, although not consistently so. Dried sporophores of *B. luteus* and *Hebeloma crustuliniforme* were also sent from Kenya to the Sudan (1960) and Ghana (1964) for nursery inoculation; the results, however, are unknown (Gibson, 1967).

A somewhat different method of spore inoculation was tried by Gilmour (1958) for chlorotic Douglas-fir seedlings in New Zealand. He made a water suspension of crushed sporophores of four species of fungi and used it for watering the seedlings; this treatment was not effective, however.

Application of sporophores, either alone or together with soil, has also been tried in the Soviet Union (Lobanow, 1960). Klyushnik (1952) inoculated surface-sterilized acorns in the autumn with spores of *Hebeloma crustuliniforme* and *Scleroderma aurantiacum* and sowed them in the spring in sterilized soil; 79 and 88% of the seedlings were mycorrhizal the following autumn, whereas only 13% of the uninoculated controls had mycorrhizae. Chastukhin (1950; see Buchholz, 1951) recommends inoculation of pine and oak seed with a spore suspension of mycorrhizal fungi for direct seeding on the steppes. The possibility of the same method is also being considered in Australia. Results of trial applications, however, are not yet available (Forrest, 1966).

For the time being, reports on the application of spores or sporophores for mycorrhizal inoculation are scattered, and hardly any results of carefully designed experiments are available. The beneficial effect of sporophore inoculation, for instance, has been reported, but often no comparison has been made with uninoculated controls. Sporophore inoculation also involves addition of a considerable amount of fresh organic matter to the soil, and therefore it is hard to decide whether the beneficial effect has been due to the inoculation itself or to the nutrient content of the inoculum.

Theoretically, the method of using spores for inoculation has many advantages, especially for shipment of inoculum for long distances. The weight and size of the inoculum would be very small and the risk of introduction of diseases could be minimized. If the spores of mycorrhizal fungi remain alive and viable for a whole year even in dry conditions, as has been reported (Lobanow, 1960), the method would be really

easy and attractive. So far, however, too little is known about the germinability of the spores of different species, as well as the factors affecting germination. More experimental work is needed before spore or sporophore inoculation on a field scale can be recommended.

IV. Introduced and Spontaneous Infection

A. Introduction of Exotic Trees Without Mycorrhizal Inoculation

In Section II numerous instances were given to indicate the necessity of mycorrhizal inoculation upon the introduction of new tree species. On the other hand, there are plenty of experiences of the successful introduction and large-scale cultivation of exotic pines and other conifers without any intentional inoculation. In South Africa and Chile, for instance, no trouble due to the absence of appropriate mycorrhizal fungi has ever been experienced, and even in those countries where mycorrhizal inoculation has later proved useful or necessary, some pines had often been grown before anything was known about mycorrhizae. Thus, in Western Australia, soil for the first inoculation was taken from an old nursery or from under an existing shelterbelt plantation (Kessell, 1927) —in other words, pines had been introduced before and grown apparently without inoculation. According to oral information (Stahl, 1967), pine nurseries were started in New South Wales and Queensland in the early 1920's and good seedlings were raised in many of them without deliberate inoculation. Likewise in Rhodesia, pines were introduced as long ago as 1900 and several thriving plantations were established in subsequent decades (Streets, 1962), although mycorrhizal inoculation was only started and its importance recognized as late as 1928 (Anonymous, 1931). Similar examples can be taken from many other countries. Even in Puerto Rico, where the history of mycorrhizal inoculation is especially well documented, three individual pine trees of the early trials survived and were never inoculated (Vozzo, 1967).

Examples of this kind are often taken as evidence that mycorrhizae are not important or necessary to exotic pines. It is most unlikely, however, that any pine can grow in natural surroundings to an age of several years without mycorrhizal association. If sowings or plantations of pine have been successful without inoculation, this does not mean that the trees are nonmycorrhizal. Closer examination has invariably shown the trees to be mycorrhizal. The question then arises as to how the trees have acquired the mycorrhizal infection.

B. Modes of Transportation of Mycorrhizal Infection

In most countries where pines or other ectotrophic trees are not indigenous, mycorrhizal fungi probably first arrived in the roots of potted plants. According to historians, early settlers often brought trees of their home countries and planted them around their new homes (see E. L. Stephens and Kidd, 1953a; Pryor, 1958). Thus, probably large numbers of living mycorrhizal seedlings of pine, oak and other European trees were planted in South Africa, Australia, New Zealand, and South America as much as 200 or 300 years ago. In those days there were no plant quarantine regulations restricting the import of living plants.

Probably mycorrhizal fungi have also arrived in many countries through botanical gardens. According to the records, the first specimens of many exotic trees were brought to the gardens as potted plants. *Pinus radiata,* for instance, was first introduced to Australia in 1857, when potted seedlings of this species were brought from Kew Gardens to the botanical gardens of Sydney and Melbourne (Fielding, 1957).

Mycorrhizal infection may also have arrived as spores attached to imported seed. In general, if seed is extracted from the cones in special extraction plants, as is customary with coniferous seed in temperate countries, there is little possibility of its becoming contaminated with spores of mycorrhizal fungi. In tropical and subtropical countries, however, it is customary to extract pine seed on the ground in direct sunshine right in the woods, where the seed can easily become contaminated with both fungal spores and mycorrhizal soil. Acorns are often collected directly from the ground. Formerly there were no quarantine regulations to require disinfection of imported seed. So far no definite proof is available that a mycorrhizal fungus had arrived anywhere as spores attached to imported seed. Some observations, however, strongly support such an assumption. Thus, in Argentina, sporophores of the North American mycorrhizal fungus *Boletus auripolus* have been found only in an experimental plantation of the hybrid pine *Pinus radiata* × *P. attenuata,* which had been raised from seed obtained from Placerville, California (Takacs, 1967b). Contamination of seed with fungal spores might also explain the occurrence of *B. elegans* under larches in remote localities where, as far as is known, living larch seedlings or soil from larch stands never have been imported (Stahl, 1968).

Very little is known about the viability and germination of the spores of mycorrhizal fungi. It is a well-known fact that spores of many mycor-

rhizal species, in general, are very difficult or even impossible to germinate on synthetic media in the laboratory (Fries, 1943; Melin, 1962). But, on the other hand, numerous experiences indicate the ease with which mycorrhizal infection spreads (Robertson, 1954). It spreads, in fact, with such ease that special precautions are usually necessary to protect experimentally grown nonmycorrhizal seedlings from air-borne infection. Australian foresters tell that mycorrhizal infection spread very rapidly after the rainy year of 1927, when the fruiting bodies of mycorrhizal fungi were extremely plentiful. The possibility of spore infection also depends on the longevity of the spores. If spores can remain viable for over a year in the dry condition (Chastukhin, 1950), the chances of air-borne infection in nurseries are greatly increased. The probability of spore infection decreases, of course, along with increasing distance from existing forests or plantations, but, at least theoretically, such a possibility can nowhere in the world be completely eliminated. It is often impossible to determine with certainty the origin of mycorrhizal infection in nurseries that have not been intentionally inoculated. In countries where inoculated nurseries and plantations already exist, infection may be transported to new ones through spore flight or other means, for instance, in soil adhering to tools, car tires, or shoes. Living plants, however, may be the most common source of infection. Potted pine seedlings have often been brought into new nurseries for ornamental purposes, or sometimes nursery sites have previously been used as staging posts of planting stock. In fact, a new nursery has seldom been established without bringing in some living seedlings from other nurseries. Thus, although records on intentional inoculation may be missing, there are numerous possibilities and sometimes even traceable evidence of an unintentional introduction of mycorrhizal infection.

C. Indigenous Mycorrhizal Fungi

Experiences illustrating the necessity of mycorrhizal inoculation have hitherto been confined mainly to exotic pines in tropical and subtropical countries and to a lesser extent to other genera of the same family (*Larix, Picea, Pseudotsuga*), and also to some other highly mycotrophic trees, such as oaks. Recent studies have shown, however, that many indigenous trees of the respective areas also have ectotrophic mycorrhizae, and accordingly there are indigenous fungi forming ectotrophic mycorrhizae. Thus, a new question arises, whether or to what degree these indigenous fungi are able to form mycorrhizal associations with introduced pines or other exotic trees.

Second to Pinaceae, Fagaceae is the best known ectotrophic tree family, all species of which probably have ectotrophic mycorrhizae. Thus, ectotrophic mycorrhizae are known on all the *Nothofagus* species of New Zealand (Morrison, 1956), and South America (Singer, 1963b, 1964) and on tropical oaks (*Quercus*) as well as in the large allied family Dipterocarpaceae (K. G. Singh, 1966). Another large group of ectotrophic trees is the genus *Eucalyptus* (Levisohn, 1958a; Chilvers and Pryor, 1965; Anderson, 1966). *Eucalyptus* deserves special attention, because pines have been introduced to Australia, the natural range of *Eucalyptus*, and conversely, eucalypts have been planted on a large scale as exotics in all the tropical and subtropical continents.

Recently, the common occurrence of ectotrophic mycorrhizae has been discovered in the family Cesalpiniaceae, comprising numerous species in both savanna and rain forests of the tropics (Peyronel and Fassi, 1957, 1960; Redhead, 1958; Fassi and Fontana, 1961, 1962; Jenik and Mensah, 1967). It is a question of primary interest whether mycorrhizal fungi of these trees can also infect pines, since in the tropics pines are usually planted in savanna woodlands, the original forest cover of which mainly consists of species of Cesalpiniaceae (*Julbernardia, Brachystegia*, etc.).

Studies on the ectotrophic mycorrhizae of the above-mentioned trees have mainly been restricted to morphological descriptions. Attempts to isolate the symbionts from the mycorrhizae have not been successful, and very few reports on aseptic syntheses of known species of trees and fungi have been published. Thus, conclusions about the mycorrhizal habit of certain fungi are usually based on field observations of their parallel occurrence with certain trees and on their taxonomic relationship to known mycorrhizal species. The substantial work of Singer (1950, 1963a,b, 1964) and his associates (Singer and Morello, 1960; Singer and Moser, 1965) must be particularly noted in this respect.

In general, mycorrhizal fungi are not particularly specialized in regard to their host plants (see Trappe, 1962a). Many of the best known and most thoroughly studied species usually form mycorrhizae with several tree species, and even with both conifers and broad-leaved trees. Thus, *Amanita muscaria* has been experimentally proved to form mycorrhizae with several species of *Pinus, Picea,* and *Betula,* and is suspected to do so with other genera too. The most cosmopolitan mycorrhizal species, *Cenococcum graniforme*, is known to form ectotrophic mycorrhizae with plants of 25 genera, including *Nothofagus* and *Eucalyptus* (Trappe, 1964).

The best known ectotrophic mycorrhizal genus of temperate forests is *Boletus* (in a broad sense). Most *Boletus* species of Europe and North America are symbiotic with forest trees, and consequently we may presume that indigenous *Boleti* of other continents, if there are any, are mycorrhizal with some local trees and potential symbionts for introduced exotics too. The same may be true of other mycorrhizal genera, such as *Russula, Lactarius, Cortinarius,* and *Tricholoma.* Some potentially mycorrhizal fungi, as *Laccaria laccata, Paxillus involutus,* and *Thelephora terrestris,* have a very wide geographic distribution or are represented by closely related species. Consequently, trees grown far outside their natural range may still meet their natural symbionts there.

Palm (1930) already noticed that an indigenous *Boletus* (*B. pallidus*?) was always associated with *Pinus merkusii* in natural stands. He also noticed the parallel occurrence of indigenous pines and *Boleti* in Guatamala.

Thanks to the extensive work of Singer and his associates (see references above), the ectotrophic flora of South America is known fairly well. *Boletus loyo,* for instance, is presumably mycorrhizal with several species of *Nothofagus* and potentially may enter into association with introduced pines too. The South American forest flora includes numerous species of *Amanita, Cortinarius, Lactarius, Russula, Tricholoma,* and other mycorrhizal genera. Species of these genera are found, for instance, in the native oak forests of Colombia (Singer, 1963b). *Paxillus involutus,* which is very common in Europe and North America, is also indigenous in Chile, whereas local *Laccaria* species are closely related to the cosmopolitan *Laccaria laccata* (Singer and Moser, 1965). It is not surprising, therefore, that the introduction of exotic pines and other conifers into Chile has apparently not been hampered by any mycorrhizal difficulties, as far as is known.

Very little is known about the indigenous fungal symbionts of the Australian eucalypts. *Pisolithus tinctorius* is regarded as one of them and is believed to have been introduced into South Africa with the eucalypts (E. L. Stephens and Kidd, 1953b), although this species is also known as an indigenous mycorrhizal fungus of pines in North America (Schramm, 1966) and elsewhere. The parallel occurrence of eucalypts and *P. tinctorius* was originally pointed out by Smith and Pope (1934). The *Eucalyptus* forest soils of South Australia contain indigenous fungi that are able to form mycorrhizae with *Pinus radiata,* although the population density of these fungi may be low (Bowen, 1963).

According to Bowen (1966), Ashton (1956) has experimentally

demonstrated that six (local?) fungi form mycorrhizae with *Eucalyptus;* these are *Cortinarius ochraceus, C. radicatus, Clitocybe australiana, Mesophellia arenaria, Agaricus xanthoderma,* and *Naematoloma fasciculare.* The inclusion of the last two in the list is surprising, since those species are usually regarded as saprophytes. *Scleroderma verrucosum,* which has been reported to be mycorrhizal with eucalypts in Israel (Levisohn, 1958a) and India (Bakshi, 1966), is a cosmopolitan species, and indigenous in both the above countries and in Australia (Dring and Rayss, 1963).

Anderson (1966) found sporophores of many local fungi (species of *Amanita, Boletus,* and *Tricholoma*) in *Eucalyptus* plantations in Italy, probably forming mycorrhizae with *Eucalyptus.* From semiaseptic synthesis experiments, Rambelli (1962a,b) considers *Tricholoma tigrinum* and *Lycoperdon gemmatum* to be mycorrhizal with eucalypts in Italy. If indigenous Italian fungi can form mycorrhizae with eucalypts, then correspondingly the Australian symbionts of *Eucalyptus* may be equally able to establish root symbiosis with introduced pines. This possibility has also been suggested by Björkman (1966).

Very little is known about the mycorrhizal fungi of the tropical families Cesalpiniaceae and Dipterocarpaceae. The fungal flora of tropical forests, however, includes numerous species of the same genera, which are known to be mycorrhizal in temperate countries. Several species of *Boletus* and *Lactarius,* for instance, have been described from the *Gilbertiodendron* forests of the Congo (Robyns, 1935–1966), and the same fungi may possibly form mycorrhizae with introduced tree species too. Duff (1936) also used "a local *Boletus sp.*" and some other local fungi for experimental inoculation of pines in Zambia. The experiments of Olatoye (1966), however, strongly support the theory that indigenous fungi of savannas and rain forests cannot infect introduced pines, at least without some kind of activation.

D. Introduced Mycorrhizal Fungi

Although mycorrhizal association may possibly be established between introduced trees and indigenous fungi, mushrooms fruiting in exotic plantations do not usually belong to the indigenous flora of the respective area.

The history of the arrival of new fungal species in various countries has not been well documented. As mentioned above, potted seedlings with mycorrhizal roots were often transported from one country to another hundreds of years ago, and, consequently, the commonest my-

corrhizal fungi were introduced to many countries long before anything was known about mycorrhizae. More recently, some few local lists of exotic fungi in coniferous plantations have been published, and, in addition, there are numerous references to the exotic nature of individual species. With regard to the fungal species, however, such references may be unreliable, since they have usually been made by foresters or other amateurs, whereas competent taxonomists are needed for reliable species determination.

Kessell (1927) already pointed out the common occurrence of *Rhizopogon luteolus* sporophores in old pine plantations and even in pine nurseries. Purnell (1957) lists the following fungi, which are almost invariably met with in pine plantations in Victoria and presumably have all been introduced to Australia from Europe:

Lactarius deliciosus
Amanita muscaria
Boletus luteus
B. granulatus
Rhizopogon luteolus

These same fungi occur in exotic pine plantations almost everywhere in Australia and also in other continents. Only a few species need to be added to the list. All the above fungi are known in pine plantations in Tasmania, and, in addition to them, *Rhizopogon roseolus* and a species of *Russula* and of *Tricholoma* (Titze, 1967). Essentially the same list was given by Stahl (1967) for the Australian Capital Territory, where two other species of *Boletus* are also known: *Boletus elegans* under *Larix leptolepis* and an unidentified (North American?) *Boletus* under *Pseudotsuga* (Stahl, 1968). *Boletus bovinus* has been reported from New South Wales and *B. edulis* from Queensland.

The first list of the fungi of exotic plantations in New Zealand was published by Birch (1937), and this has later been extended by Rawlings (1950, 1958). *Amanita muscaria, Boletus luteus,* and *B. granulatus* are the most conspicuous species in New Zealand. Notably *Lactarius deliciosus* is lacking. Rawlings mentions *Boletus elegans* under *Larix, B. scaber* under *Betula,* and *B. lakei* under *Pseudotsuga. Boletus piperatus, Scleroderma bovista* and a *Tricholoma sp.* are also found under both pine and Douglas fir. *Laccaria laccata* (or some closely related species) occurs in both New Zealand and Australia, where it may even be indigenous. Detailed studies on indigenous and exotic *Boleti* of New Zealand have recently been published by McNabb (1967, 1968).

The mushroom books of E. L. Stephens and Kidd (1953a,b) include numerous fungi that were originally imported to South Africa with potted tree seedlings and now commonly grow in exotic plantations and parks. *Amanita muscaria*, *A. rubescens* and *A. pantherina* are common in pine plantations, whereas the deadly poisonous *A. phalloides* seems to be associated with oaks. *Lactarius deliciosus* is so characteristic of pine plantations that it has the local name "pine ring." *Hebeloma crustuliniforme* is also known in South Africa. Both *Boletus edulis* and *B. bovinus* are common in pine plantations, and *B. duriusculus* under poplars. A most interesting feature is the common occurrence of *B. bellini* in South Africa, because, according to Reichert (1940b), *B.* (*Rostkovites*) *bellini* is a strictly Mediterranean species and always associated with *Pinus halepensis*.

Pisolithus tinctorius is the predominant fungus under *Eucalyptus* in South Africa and is supposed to have arrived with potted seedlings from Australia, although it is also indigenous in both Europe and North America. In Israel *P. tinctorius* is an exotic species, also introduced with *Eucalyptus* (Reichert and Avizohar-Hershenzon, 1959).

The commonest mushrooms in Usutu Forest of Swaziland in March 1967 were (Mikola, 1968):

Laccaria laccata
Amanita muscaria
Boletus edulis
Rhizopogon roseolus

The following species were also observed:

Boletus granulatus
B. luteus
B. piperatus
Hebeloma crustuliniforme (?)
Tricholoma sp.
Thelephora terrestris

According to Gibson (1963, 1967), *Boletus luteus* is the predominant mushroom in East African pine plantations. *Scleroderma bovista* is also known in Kenyan and Tanzanian plantations, whereas a *Rhizopogon sp.* has been met with only once in a nursery. *Hebeloma crustuliniforme* was recorded for the first time in 1959 and is now common in several nurseries, fruiting in transplant beds. *Laccaria lateritica* was first found in 1966 in a nursery. *Thelephora terrestris* is a very common fungus in

nurseries and plantations in both Kenya and Uganda (Brown, 1963). *Amanita muscaria,* one of the most common exotic fungi of South African pine plantations, has not been found in East Africa, although it occurs in Rhodesian plantations (Gibson, 1967).

Sporophores of *Rhizopogon* are rather common under pines (*Pinus oocarpa, P. caribaea* and *P. pseudostrobus*) in Nigeria, even 1 to 2 years after planting. The morphology of the mycorrhizae suggests the presence of *Boletus* spp. too (Mikola, 1968).

Observations of the same kind have been made in Central and South America (Mikola, 1968, and communications from Singer, 1967; Takacs, 1967b; Cardoso May, 1967, and others). *Boletus granulatus* and *B. luteus* are common in pine plantations in Chile, Argentina, and Brazil, whereas in the most tropical areas (Trinidad and Puerto Rico) *Boletus* spp. are so far probably lacking. *Boletus luteus* is apparently more common in cool and humid areas (Chile and Southern Argentina), whereas *B. granulatus* also thrives in warm and dry conditions (cf. Singer, 1963a), being, for instance, the only species fruiting in the *Pinus halepensis* plantations of La Pampa (Piterbarg, 1967). *Boletus elegans* is almost invariably found under *Larix* in both Chile and Argentina. *Boletus bovinus* is reported from Argentina. *Lactarius deliciosus* is also common in Chile and Uruguay. *Amanita muscaria* is common in Chile, where it can probably also infect indigenous *Nothofagus* trees, but it is uncommon in Argentina (found under *Cedrus deodara* in Bariloche) and has not yet been found in the São Paulo region of Brazil. A yellow *Amanita* was also found under pine plantations in Chile. *Amanita phalloides* is common under oaks in Argentina (Singer, 1950) and probably has been introduced with potted seedlings of park trees from Europe in the same way as it was to South Africa. *Laccaria laccata* (or some closely related species) often appears in plantations and may also be indigenous.

Puffballs (*Rhizopogon, Scleroderma,* and *Pisolithus*) are also common associates of pines in Central and South America. *Thelephora terrestris* is the most common fungus of pine plantations in Puerto Rico and Trinidad. Sporophores of *Pisolithus tinctorius* now occur under pines in Surinam where mycorrhizal soil has been brought from British Honduras (van Suchtelen, 1962).

There are examples of the introduction of mycorrhizal fungi to Europe too. Beside the well-known association of *Boletus elegans* with larch, wherever any *Larix* species are cultivated as exotics, larch also has other specialized symbionts which have followed their host to new areas (Kreisel, 1963). *Boletus amabilis,* which appears under Douglas-fir

plantations in Central Europe, has arrived from the natural range of the Douglas-fir in North America (Moser, 1967).

Introduced mycorrhizal fungi may sometimes be of considerable economic importance, improving the economy of exotic plantations. Many of the common introduced species, such as *Lactarius deliciosus* and *Boletus edulis* and other *Boleti,* are edible, and when mushrooms grow in masses under plantations they constitute an important source for the canning industry. Their value is increased by the fact that apparently the parasites of the mushroom were not always introduced with the hosts. Thus, for instance, in Australia and South America sporophores of *L. deliciosus* are always clean, whereas in Europe it is almost impossible to find a specimen that is not full of "worms."

E. Specificity and Adaptability of Mycorrhizal Fungi

In general, mycorrhizal fungi are not exacting with regard to their host species. It is most probable, for instance, that some indigenous mycorrhizal fungi of *Nothofagus* spp. have formed mycorrhizae with introduced pines in New Zealand and South America. On the other hand, in the natural environment, competition must occur between different species of mycorrhiza-forming fungi. Consequently, those fungal species best adapted to the particular host species and the prevailing conditions gain dominance and the others disappear. Thus, if a tree species is introduced to a new area without its usual mycorrhizal symbionts, it may first develop mycorrhizae with some indigenous fungi. Later, however, these may be replaced by more specific symbionts, which are introduced intentionally or arrive incidentally. In such cases the first colonizers of the roots are either specific mycorrhiza-formers of indigenous trees or ubiquitous facultative fungi, which can also live saprophytically in forest soils. With regard to pines in Chile, for instance, *Boletus loyo* may belong to the first group (obligate mycorrhiza-formers with *Nothofagus*) and *Laccaria* spp., *Thelephora terrestris,* and *Paxillus involutus* to the latter. Apparently, however, *Boletus granulatus, B. luteus, Lactarius deliciosus* and other indigenous fungi of the pine forests of Europe and North America are better adapted to pines, and therefore these fungi predominate today in exotic pine plantations throughout the world.

We may also presume that these specific "pine fungi" are more efficient symbionts, i.e., more beneficial to pines, than ubiquitous facultative species. Such an assumption is strongly supported, for instance, by the studies of McComb and Griffith (1946) in Iowa, where potential mycorrhiza-formers were apparently present in treeless grassland soils.

In natural soil, however, they were inactive and could be activated only with phosphorus fertilization. On the other hand, soil inoculation with the fungal population of pine forests greatly stimulated the formation of mycorrhizae and the growth of seedlings without any additional fertilization.

Laccaria laccata and *Thelephora terrestris* are mentioned above as examples of ubiquitous facultative mycorrhiza-formers, which are probably often the first fungi to infect exotic conifers, until more specific symbionts are introduced. Both of these fungi were considered typical soil saprophytes until their mycorrhizal habit was demonstrated quite recently (W. C. Bryan and Zak, 1961; Meyer, 1963; Hacskaylo, 1965; Fassi and Fontana, 1966). *Thelephora terrestris*, in particular, seems to be one of the first mycorrhizal colonizers of new nursery sites, and predominates in many nurseries (Zak and Marx, 1964). It is often a real nuisance in greenhouses and nurseries, preventing the raising of non-mycorrhizal seedlings for experimental purposes. In some new nurseries (in Australia and Brazil) it has even been harmful, smothering young seedlings, but later on it has usually disappeared, probably because of the increasing population of competing fungi. *Thelephora* was also the first fungus whose sporophores appeared in pine plantations in Puerto Rico.

Although most known mycorrhizal fungi can infect a great number of tree species, others are more specialized with regard to their hosts. The best known example is *Boletus elegans*, which is found only under *Larix* species. Some other fungi, too, seem to be confined exclusively to larch (Kreisel, 1963). *Amanita phalloides*, at least when growing as an exotic, seems to depend on oaks (E. L. Stephens and Kidd, 1953b; Takacs, 1967b). Other trees may have the same kind of specialized symbionts, although they are not so well known (see Trappe, 1962a). Logically, we may assume that such specific mycorrhizal associates are also more efficient symbionts than the ubiquitous cosmopolitans; at least *Boletus elegans* is probably the best symbiont for *Larix*, *B. plorans* for *Pinus cembra* and *B. lakei* (*B. amabilis*) for *Pseudotsuga*.

Fortunately, *Boletus granulatus* and *B. luteus*, the most constant companions of pines in exotic plantations, are probably quite good symbionts. It is not known, however, whether the same species are the best associates for spruce and Douglas-fir and other conifers as well. In fact, several examples suggest quite the opposite. Pryor (1958), for instance, suspects that the poor growth of spruces in Australia may be

due to the absence of appropriate mycorrhizal fungi. Several fungi in Australia form mycorrhizae with spruce, but the most efficient species may still be lacking. Rawlings (1958) likewise assumed that the best mycorrhizal symbionts of spruce and Douglas fir were still lacking in New Zealand, and suggested the introduction of *Lactarius deliciosus*. Inoculation has sometimes improved the growth of Douglas fir in New Zealand, although the inoculum for those experiments was obtained locally (Gilmour, 1958).

An example from Hawaii strongly suggests the existence of specific mycorrhizal fungi of Douglas fir (L. W. Bryan, 1967). The first pines were imported into Hawaii as seedlings from California, and they probably brought mycorrhizal fungi in their roots. Thereafter there has never been any difficulty in raising pines in nurseries without inoculation. Douglas-fir seedlings, however, grew poorly in the nurseries where pines grew well, and Douglas-fir plantations in the field also grew poorly, although they had some kind of mycorrhizae. Then, in 1936, a lot of Douglas-fir seedlings were imported from the state of Washington and immediately planted out in the field. This plantation grew well from the very start. Soil from this Douglas-fir plantation, the only one in Hawaii that has been raised from imported seedlings, was then used to inoculate Douglas-fir beds in nurseries, and since then there has been no difficulty in raising Douglas fir in Hawaii.

Different species of pine differ very little in their mycorrhizal relations. There may be, however, some differences in the susceptibility to mycorrhizal infection. To nurserymen of tropical countries, for instance, it is quite a familiar experience that seedlings of *Pinus elliottii* and *P. caribaea* turn yellow a few weeks after germination but soon resume a green color and start to grow, whereas such a chlorotic phase seldom occurs in the development of *P. radiata*. This difference is probably connected with the commencement of mycorrhizal infection. Mycorrhizal development starts earlier in *R. radiata* than in *P. elliottii* and *P. caribaea* and therefore the former species has no chlorotic stage, whereas the recovery of the green color in the latter two coincides with the development of the first mycorrhizae. The great ease with which *P. radiata* can be raised in Australia and elsewhere may also indicate its greater adaptability to mycorrhizal association with local fungi.

Pinus roxburghii (*P. longifolia*) is another example of a species which may be more capable of symbiosis with indigenous fungi than other pines (Clements, 1941). *Pinus merkusii* may represent the opposite type,

a species that does not easily become infected (van Alphen de Veer *et al.*, 1954). These speculations, however, require experimental confirmation.

Many mycorrhizal fungi are very adaptable to widely different climatic conditions, e.g., *Boletus granulatus*, which grows both in the native coniferous forests of temperate zones and in subtropical pine plantations round the world. It is really surprising to see typical fungi of the boreal forest of Europe and North America, such as *B. luteus* and *Lactarius deliciosus*, fruiting in masses in the subtropical plantations of Australia or South Africa. All fungi are not equally adaptable, however. Thus, two of the most common and most characteristic mycorrhizal fungi of the cool boreal forest, *B. luteus* and *B. variegatus*, are conspicuously different. The former grows almost everywhere in subtropical and even tropical pine plantations, whereas the latter hardly can be found outside its natural range.[3]

The adaptability of fungal species to different soil conditions also deserves attention. Ectotrophic mycorrhizal fungi are usually acidophilic, and therefore, for inoculation of alkaline soils, concomitant acidification is essential. There are, however, mycorrhizal fungi that grow naturally on calcareous soil. *Boletus granulatus* seems to be quite adaptable with respect to soil pH; it is reported to be one of the dominant species both in the natural *Pinus halepensis* forests on limestone rocks in Israel (Reichert and Avizohar-Hershenzon, 1959) and in plantations of the same species on alkaline soils in La Pampa Province in Argentina (Piterbarg, 1967). These data are surprising, because in pure-culture experiments *Boletus granulatus* has a rather narrow pH range with an optimum at 5.0 and does not grow at all at a pH above 7.0 (Modess, 1941). Probably *B. granulatus* is a collective species including several subspecies and varieties or even separate species. Thus Reichert (1940a), for instance, reported four closely related species from Israel, none of which is probably the true *B. granulatus*, whereas Trappe (1962a) considered one of these species, *B. boudieri*, synonymous with *B. granulatus*. Another species of the same group, *B. bellini*, has been reported as an exotic in South African pine plantations (E. L. Stephens and Kidd, 1953a). As a whole, the taxonomy of the mycorrhizal fungi of exotic plantations deserves intensive study in which special emphasis should be placed on the physiological and ecological properties of the fungi.

[3] According to a recent personal communication from Horak (1968), *Boletus variegatus* has been found under pine on the South Island of New Zealand.

V. Discussion

Soil inoculation may have three different purposes:

1. introduction of mycorrhizal fungi to a soil where they are totally absent
2. replacement or enrichment of the existing mycorrhiza-forming fungal population with new and more efficient species
3. improvement of soil conditions for the growth and activity of the mycorrhiza-forming fungi present

It is not known with certainty whether any natural forest or grassland soils are completely lacking in fungal species capable of forming ectotrophic mycorrhizae with introduced pines or other ectotrophic trees. Although afforestation trials failed in numerous countries before the introduction of mycorrhizal inoculum, there still remains the possibility that some potential mycorrhiza-formers were already present, but that because they were either inefficient symbionts or were in an inactive state, afforestation succeeded only after the introduction of more efficient species. The natural spread of mycorrhizal fungi seems to be very easy. It is interesting to observe that the most convincing evidence of the indispensability of mycorrhizal inoculation has usually come from remote islands, like Fiji, Puerto Rico, and Trinidad, i.e., from countries to which the chances of spore flight or of accidental transmission of infection by other means are minimal. Under such conditions artificial inoculation is most important, whereas afforestations near existing natural forests or plantations of the same species (or other ectotrophs) may succeed without inoculation, as is shown by experiments with direct seeding in Australia (Forrest, 1965). Even then, however, inoculation may be justified because of faster mycorrhization and, consequently, better survival and initial growth of the seedlings (Bowen and Theodorou, 1967).

The success of introduction of new fungi to soils where some mycorrhiza-formers are already present depends on the vigor of the indigenous fungi, the competitive capacity of the introduced species, and environmental conditions. Where the original mycorrhizal population, if any, has a low density and consists of relatively inactive species, even a small amount of inoculum is sufficient to establish uniform mycorrhization. On the other hand, if the soil already contains a dense population of mycorrhiza-forming fungi, as apparently in Austrian nurseries, for instance, a heavy application of inoculum is usually needed. In addition, modification of soil conditions may be necessary to promote the desired

type of infection. In general, specialized mycorrhiza-formers seem to be superior to any local fungi, as is shown by the constant association of *Boletus elegans* with larches or *Boletus granulatus* with pines almost everywhere in the world, under a wide range of soil and climatic conditions. On the other hand, mycorrhizal association is closely dependent on environmental conditions (Björkman, 1942, 1949). Thus, the fungal populations of nurseries usually differ greatly from those of natural forest soils, and consequently the mycorrhizal fungi which inhabit the seedling roots in the nursery are gradually replaced by other species after transplantation to the field (Adams, 1951; Mikola, 1965).

For successful inoculation, improvement of soil conditions is often essential, especially if the soil already contains mycorrhizal fungi. Pure-culture inoculation, in particular, requires special measures to guarantee the survival and thriving of the introduced fungi. Acidification and increase of organic matter are usually the most beneficial measures. In Austria, for instance, the fungal inoculum is raised on a peat nutrient substrate and large amounts of inoculum are applied to the soil, increasing the organic matter content and sometimes also the acidity. Likewise in the Soviet Union, relatively large amounts of organic matter are usually added with the mycorrhizal inoculum (Lobanow, 1960). Zerling (1958, 1960) has shown that inoculation alone is not sufficient for larch in alkaline chernozem soils. Soil acidification is also necessary and, in addition, both watering and application of some trace elements greatly increase the development of mycorrhizae and the growth of seedlings.

Soil improvement alone, without inoculation, may sometimes induce mycorrhizal development, indicating that mycorrhizal fungi are present, but owing to adverse soil conditions they are in an inactive state. Rayner (1934, 1936, 1939), for instance, used organic compost, both alone and in combination with inoculation, to establish mycorrhizal development in "sick" heath soils. Several Soviet scientists (see references in Lobanow, 1960, pp. 255–259; Puschkinskaja and Mischustin, 1963) have expressed the opinion that mycorrhizal inoculation is unnecessary, because good growth and mycorrhization of pine and oak seedlings can be achieved by activation of existing fungi by organic or inorganic fertilization and improvement of soil moisture conditions. This may be true in the forest steppe zone of the Soviet Union, where the soil probably contains some suitable mycorrhizal symbionts of oak, and spore-producing forests are not too distant. In contrast, corresponding studies conducted in the prairie forest belt of the United States (Wilde, 1954) strongly support the necessity of mycorrhizal inoculation.

Phosphorus fertilization is an effective measure for activation of mycorrhiza-forming fungi, as was clearly demonstrated by the experiment of McComb and Griffith (1946).

Undoubtedly, highly mycorrhizal tree species do not, and probably cannot, grow under natural forest conditions without mycorrhizal association. With due precaution, however, it is possible to raise nonmycorrhizal seedlings of the same species under nursery or other artificial conditions. According to personal communications, as from Puerto Rico (Wadsworth, 1967) and Nigeria (Lowe, 1967), vigorous and healthy-looking nonmycorrhizal seedlings have been raised in nurseries, but they have always died soon after being planted out into the field. According to other reports uninoculated seedlings turned chlorotic and died in the nursery. Probably, however, it would have been possible to keep them growing further by increased watering and fertilization.

Reasons for the death of nonmycorrhizal seedlings after transplantation into the field have been much discussed. The moisture factor deserves particular attention. Several authors have emphasized the importance of early mycorrhization in dry areas (Parry, 1953, 1956; Lepoutre, 1964). Mycorrhizal association, however, is in all probability indispensable for highly mycotrophic trees in areas with moist climates as well. Therefore, the question arises why the most serious difficulties due to mycorrhizal deficiency have always been experienced in areas with a pronounced dry season. The following reasons may be suggested:

1. According to the physical theory of Hatch (1937), the primary importance of the ectotrophic mycorrhizal association is in increasing the water uptake and drought resistance of the trees. Thus, if seedlings are not mycorrhizal by the beginning of the first dry season, they cannot survive. On the contrary, in moister climates the seedlings can survive longer without mycorrhizae, and therefore have better chances to become infected by either air-borne spores or indigenous soil fungi.

2. The indigenous population of mycorrhizal fungi, if any, is perhaps denser in moister climates. The longer the dry season, the greater is the probability of the mycelia dying out in the soil, whereas a continuously moist soil with a reasonably high content of organic matter offers better chances for survival.

3. Moist soils also offer better substrates for the germination of fungal spores, and, consequently greater chances for air-borne infection than dry soils.

For all the above reasons, mycorrhizal inoculation or other measures to secure good early development of mycorrhizae are particularly im-

portant in dry areas. The ecological significance of ectotrophic mycotrophy, however, has been emphasized in cold climates too. Because the climates near alpine and arctic timber lines are humid without any pronounced dry season, there must be other factors besides moisture that favor the mycotrophic mode of nutrition. Moser (1967) has suggested that mycotrophy enables the trees to use the short growing season more effectively to obtain a sufficient amount of nutrients for the long cold season and attain the necessary resistance before the early frosts. Very probably the advantage of mycotrophy in cold climates is also connected with the nitrogen nutrition of the trees. As is generally known, mycorrhizal fungi have a greater ability than their hosts to utilize complex organic nitrogen compounds, and therefore, because nitrogen mobilization is extremely slow in cold arctic and alpine soils, trees can better fulfill their nitrogen requirements in symbiosis with fungi.

The degree of mycotrophy is somewhat variable even among highly mycotrophic trees. Although probably all of them are unable to grow without fungal association under natural forest conditions, some species seem to be more exacting than others in regard to specific fungal symbionts, and there are also differences in the ease with which they become infected. Of tropical pines, according to Asian experience (Letourneux, 1957), *Pinus merkusii*, *P. khasya*, and *P. insularis* are the most dependent on early mycorrhization, whereas *P. caribaea* and *P. patula* are less dependent. *P. radiata* is usually infected more rapidly than *P. caribaea* and *P. elliottii*. *P. roxburghii* seems to be one of the least exacting pines in regard to the fungal species (Clements, 1941). Perhaps some other conifers are still more specialized than pines, for example *Pseudotsuga menziesii*, as is shown by the experiment of McComb and Griffith (1946) and the attempts at its introduction to Hawaii (L. W. Bryan, 1967). Some specific mycorrhizal requirements have also been suggested as probable reasons for the relatively poor success of spruce in Australia and New Zealand (Pryor, 1958; Rawlings, 1958). The experiments of Bergemann (1955) indicate that both *Picea* and *Larix* are probably more dependent of mycotrophic nutrition than *Pinus*.

Many ectotrophic trees are probably less mycotrophic than pines; Lobanow (1960) calls them weakly mycotrophic. There may exist a whole range of trees from nonmycotrophic species through weakly mycotrophic ones to obligate mycotrophs, the necessity of fungal association also depending on climatic and soil conditions. From the point of view of exotic plantations, *Eucalyptus* species deserve attention. In general, no mycorrhizal difficulties have been experienced when eucalypts have

been introduced into new areas and they have therefore been supposed to be totally independent of the presence of mycorrhizal fungi (Letourneux, 1957). More recently, however, ectotrophic mycorrhizae have been found on practically all species of *Eucalyptus*, although only a small proportion of the total number of short roots may be mycorrhizae (Levisohn, 1958a; Chilvers and Pryor, 1965; Anderson, 1966, 1967). As a whole, very little is so far known about the importance of mycorrhizal association for different species of *Eucalyptus* under different conditions.

Although this review is mainly concerned with ectotrophic trees and the importance of mycorrhizal inoculation for them, some reference to endotrophic mycorrhizae is necessary for comparison. In general, practical experience has shown inoculation to be unnecessary for trees with endotrophic mycorrhiza. African nursery instructions, for instance, particularly emphasize that soil inoculation is not necessary for *Cupressus lusitanica*. Cypress plantations have been successful in many areas, such as East Africa and Puerto Rico, whereas at the same time pine trials failed because of mycorrhizal deficiency. The obvious reason for the nonessentiality of inoculation of endotrophic mycorrhizae must be the presence of appropriate fungi practically everywhere. *Endogone* species, the probable symbionts of vesicular-arbuscular endotrophic mycorrhizae, seem to be quite cosmopolitan in regard to both geography and host species.

Mycorrhizal fungi can survive for a long time in soil even without tree vegetation. Wilde (1954) has expressed this in the statement "once forest soil, forever forest soil." There is usually no need for inoculation of new nurseries on former agricultural soils if the area has once been forested with ectotrophic trees. With prolonged agricultural use, however, especially if lime has been applied, some impoverishment or undesirable selection of the mycorrhizal population may have taken place, and therefore inoculation is recommended for soils which have long been under agriculture (Wakeley, 1954; Stoeckeler and Jones, 1957). If some trouble appears in new nurseries on old agricultural soils, mycorrhizal deficiency may always be suspected. As experience in Austria shows, enrichment of the mycorrhizal population and introduction of new species may be both feasible and advantageous. Some improvement of soil conditions, usually acidification, is often necessary at the same time.

Soil sterilization is a common practice in modern nursery management, and its influence on mycorrhizal fungi deserves particular attention. Since a harmful effect is probably to be expected, several experi-

mental studies have been conducted (Wilde and Persidsky, 1956; Hacskaylo and Palmer, 1957; Persidsky and Wilde, 1960; Laiho and Mikola, 1964). These studies have consistently shown that fungicides usually delay the commencement of mycorrhizal infection but do not prevent it entirely. The lag varies, depending on both the type of fungicide and the strength of application. Some cases, however, have been reported from both New Zealand and the United States (Bassett, 1967; Davey, 1967) in which soil sterilization had destroyed the mycorrhizal population and resulted in stunted, chlorotic seedlings. In these cases the seedlings were saved and cured by soil inoculation. In nurseries where soil sterilization is a regular practice, the delay of mycorrhizal infection may be disastrous if the seedlings are still nonmycorrhizal at the time of lifting. Therefore, the development of root systems must be repeatedly inspected in the nurseries, and mycorrhizal inoculation should be performed if needed. Mycorrhizal development also deserves special consideration in the grading of nursery stock (Jorgensen and Shoulders, 1967). The use of unsterilized pine-needle litter as mulch, as is customary in many nurseries of the southern United States, is based on sound reasoning, the mulch functioning at the same time as mycorrhizal inoculum.

Soil sterilization can also improve the conditions for mycorrhizal infection. Sterilization reduces competition, and if a suitable source of infection is present, mycorrhizal development may be much faster than in unsterilized soil. Thus, in Zambia, seedlings are raised in sterilized soil in polythene tubes, and mycorrhizal development is very vigorous without any inoculation, the soil beneath the tubes being the probable source of infection. It is also common experience that *Thelephora terrestris* very rapidly colonizes sterilized soil in greenhouses and forms masses of sporophores, whereas it is not so conspicuous in unsterilized soil. The effect of soil sterilization appears to be quite complicated. Several studies have shown, for instance, that sterilization with certain chemicals, such as formaldehyde and allyl alcohol, strongly favors the overwhelming dominance of the soil mold *Trichoderma viride*, which, in turn, seems to promote mycorrhizal fungi.

Our ignorance of the efficiency of different species as symbionts, and the technical difficulties of the procedure, have been the main obstacles to pure-culture inoculation. Even at the present stage of knowledge, however, it would be possible to take more systematic action toward mycorrhizal inoculation (Singer, 1963a). Selection of inoculating material for warm and dry areas as well as for alkaline soils deserves particular attention.

Until more is known about the ecological properties of individual fungal species, the most reliable method is to procure inoculum from areas with conditions corresponding as nearly as possible to the conditions of the area where the inoculum will be used. Of individual species, *Cenococcum graniforme* is known to be especially drought resistant (Worley and Hacskaylo, 1959; Trappe, 1962b) and might be used for inoculation in particularly dry conditions. Its efficiency as a symbiont, however, is somewhat questionable. Preliminary experiments with inoculation with *Cenococcum* in the tropics have failed (Vozzo, 1967), whereas inoculation in the temperate climate has been successful, at least under certain conditions (Mikola, 1955). Mycorrhizal fungi also have very variable temperature requirements and even local strains of the same species may differ in this respect (Moser, 1958d). Sensitivity to high temperatures may be the reason for the absence of *Boletus variegatus* in subtropical pine plantations. *Pisolithus tinctorius* is probably one of the most suitable species for tropical conditions. It has relatively high temperature requirements (Marx, 1966) and grows naturally in the tropics; in the temperate zones it is often found growing on extremely warm sites, such as the black waste banks of coal mines (Schramm, 1966). *Thelephora terrestris* is another species that is adaptable to warm climates; its symbiotic efficiency, however, is so far unknown, as well as the technique of growing pure cultures. Selection of suitable mycorrhizal fungi may be of great importance in afforestation of exceptionally cold areas also (Moser, 1956).

Ectotrophic mycorrhizal fungi are usually acidophilous, and acidification of alkaline soil is often an indispensable requisite for successful inoculation or an effective measure to stimulate existing fungi. There are numerous ectotrophic tree species (e.g., *Pinus nigra* and *P. halepensis*, *Quercus* spp.), however, which prefer an alkaline substrate and form forests on limestone rocks. In all probability these forests contain a natural population of basophilous mycorrhizal fungi. The physiological and ecological properties of these fungi, as well as their pure culture technique, are poorly known and should be studied more thoroughly. Meanwhile, soil of natural *P. halepensis* forests can be used as inoculum in alkaline areas.

VI. Recommendations

A. Forest Research

As is apparent from the above review, the application of mycorrhizal inoculation in forestry practice is based mainly on hypotheses and field

experience. Relatively little is known with certainty. In view of the tremendous afforestation programs in the tropics and subtropics, there is an urgent need for research to give a reliable scientific foundation for practical work. Below are outlined some topics needing research in the immediate future:

1. *Comparative Studies on the Physiology, Ecology, and Symbiotic Efficiency of Different Fungal Species.* Progress on this line of research would provide a concrete foundation for the selection of species for pure-culture inoculation and for a biologically sound nursery soil management. It would be most desirable to find suitable mycorrhizal fungi for exceptional conditions, such as alkaline or extremely acid soils, permanently or temporarily water-logged soils, and dry or hot climates.

For the study of the above problem, seedlings inoculated with pure cultures should be grown under field conditions. Such experiments can be conducted only in virgin areas, where soils are likely to be free of mycorrhizal fungi. Unfortunately, because of the indiscriminate importation of soil and living plants, soils of most countries are already so badly contaminated that relatively few areas are any longer available for this kind of experimentation.

2. *Development of Practicable Techniques of Pure-Culture Inoculation.* With a pure-culture technique it would be possible to favor the most efficient species and to avoid the less efficient and harmful ones, and the risk of introducing pests would be avoided.

3. *Development of a Technique of Seed Inoculation.* In many countries afforestation programs are so vast that they are hard to carry through by planting alone. Application of direct seeding would greatly facilitate this work, provided the commencement of mycorrhizal development could be assured.

4. *Further Studies on Mycorrhizal Relations in the Poorly Known Ectotrophic Trees.* The most important of these are members of the genera *Eucalyptus* and *Nothofagus.* The group also includes other tropical and subtropical species of Fagaceae, and the families Dipterocarpaceae and Cesalpiniaceae. The fungal symbionts should be studied, the mycorrhizal habit should be confirmed with synthetic cultures under aseptic conditions, and cross inoculation experiments should be conducted, i.e., inoculation of pines, oaks, etc., with mycorrhizal fungi of *Eucalyptus, Nothofagus* and other ectotrophic trees of the tropics and southern hemisphere, and *vice versa.* In this way more light could also be thrown on the complex problem of obligate and facultative mycotrophy.

5. *Endotrophic Mycorrhizae of Forest Trees.* Although the problems of practical silviculture have been mainly concerned with ectotrophic mycorrhizae, the great majority of forest trees have the endotrophic type of root symbiosis. Endotrophic mycorrhizae represent a more intimate form of symbiosis and greater diversity of physiological relationships than the relatively uniform ectotrophic mycorrhiza (see Mosse, 1963). Recent studies have even given some indication of nitrogen fixation in some endotrophic mycorrhizae (Becking, 1965).

6. *Geographic Distribution and Migration History of Exotic Mycorrhizal Fungi.* Although this field of study may not be of great practical importance to afforestation, phytogeographically and ecologically it is a problem of primary interest. In countries where afforestation with exotics is young or just beginning, it is eminently desirable that records of the migration of exotic fungi should be kept up to date, whereas in those countries where exotic plantations date back for a century or more, the early migration of fungi may be impossible to trace by now and only the present situation can be documented. A worldwide survey of the present distribution of *Amanita muscaria, Boletus edulis, B. luteus, B. granulatus* and *Lactarius deliciosus,* for instance, would make a good starting point for continued studies.

B. Forestry Practice

In forest countries where ectotrophic trees and associated fungi are indigenous, there is little need or possibility for mycorrhizal inoculation. If troubles connected with poor development of mycorrhizae appear in a nursery, a remedy can probably be achieved more readily by improving the soil conditions (acidity, fertility, aeration, or content of organic matter) than by introduction of new fungi. Cases in which inoculation is beneficial and practicable, as in the afforestation of alpine areas in Austria, are still exceptions. It is always possible, however, that in the future research will reveal new ways of deriving benefit from mycorrhizal inoculation even in forested countries.

In areas where exotic trees have grown for a considerable time and soils are well infected with mycorrhizal fungi the situation is almost the same. There, mycorrhizal inoculation is usually both unnecessary and useless, but because the density of mycorrhizal populations in the soils may be low, at least locally, some safety measures may be advisable to guarantee early mycorrhization of the seedlings.

Soil is usually brought to tropical and subtropical nurseries from natural forests, plantations or grasslands. The common practice of adding

10 to 20% of pine soil to the soil mixture for pots, tubes, trays or Swaziland beds is not necessary in old nurseries, which are already well infected with mycorrhizal fungi. Today, nurseries are often located in the midst of pine plantations, and in such cases it is most practicable to bring all the soil needed in the nursery from these plantations. If the soil is poor in nutrients, this can easily be corrected with commercial fertilizers. A moderate nutrient level is also necessary for good mycorrhizae. Mycorrhizal development is often best promoted by phosphorus fertilization.

Soil for seedbeds and transplant pots is often sterilized in tropical and subtropical nurseries. Even then inoculation has usually proved unnecessary in old nurseries, but some kind of guarantee of mycorrhization is advisable. Early mycorrhization is promoted with unsterilized pine litter mulch or by soil inoculation.

Inoculation of new nurseries is recommended, although they may also become infected naturally if old nurseries or plantations are not too far away and especially if there is traffic between old and new nurseries. Artificial inoculation assures a more rapid and uniform infection of the whole nursery. Soil inoculum may be used if it can be obtained locally from a healthy and vigorous plantation. Soil of a healthy forest plantation is always preferable to nursery soil, since the latter is more likely to contain propagules of damping-off or other diseases. For longer distance transportation, richly mycorrhizal potted plants are preferable; their roots are finely chopped and mixed with the potting soil. For *Pinus merkusii*, however, present experience advocates the use of mother trees. *Eucalyptus* (the renantherous species perhaps being an exception), cypress, and many other genera do not need any inoculation.

For the importation of mycorrhizal inoculum from abroad, pure cultures are preferable (Bakshi, 1967). The same method is also advisable for long-distance transfers inside countries. Although the technique of pure-culture inoculation is poorly mastered, some recommendations can already be made (Moser, 1958b,c, 1959). Regarding the fungal species, very little is known with certainty. In all probability, however, *Boletus granulatus* is one of the most efficient species for pines, perhaps the most efficient one, and very adaptable to widely different ecological conditions. For alkaline soils, the Mediterranean variety of *B. granulatus* should be isolated and cultivated, as well as the other related Mediterranean species of *Boletus*. At the present stage of our knowledge, however, it is advisable to use a mixture of several species for pure-culture inoculation.

If, however, pure-culture inoculation fails, then importation of soil or living seedlings is necessary. Soil should be taken from conditions as similar as possible to those of the area to be afforested. To reduce the risk of introducing diseases, only soil from healthy natural forests should be considered and only the necessary amount should be brought. Soil can also be treated with insecticides which do not harm fungi.

Mycorrhizal seedlings are probably still safer than soil. Healthy seedlings can be removed from the soil, the roots washed and only seedlings with good mycorrhizae accepted. Thick and profusely branched dichotomous mycorrhizae with a white mantle are characteristic of thriving and vigorous pine seedlings. Shoots can be treated with pesticides, or it is possible to remove the shoots and send only the mycorrhizal roots in moist soil for use in inoculation.

Plant quarantine regulations should be adjusted to make legal importation of mycorrhizal inocula possible.

REFERENCES

Adams, A. J. S. (1951). A forest nursery for *Pinus radiata* at Mt. Burr in the south-east of South Australia. *Aust. For.* **15**, 47–56.

Allan, T. G., and Endean, F. (1966). Manual of plantation techniques. *For. Dept. Gov. Zambia* (Stencil.).

Anderson, J. (1966). Indagini sulla micorrizia in alcune specie di eucalitto nell'Italia Centrale. *Pubbl. Cent. Sper. agric. for., Roma* **8**, 259–274.

Anderson, J. (1967). Le micorrize dell'eucalitto in piantine allevate in vivaio. *Pubbl. Cent. Sper. agric. for., Roma* **9**, 89–90.

Anonymous. (1931). Establishing pines. Preliminary observations on the effect of soil inoculation. *Rhod. agric. J.* **28**, 185–187.

Anonymous. (1947a). Mycorrhiza on seedling pines grown in uninoculated soil. *Rep. For. Dep., Nyasald.*

Anonymous. (1947b). *Pinus caribaea* in Trinidad. *Rep. For. Dep., Trinidad and Tobago.*

Anonymous. (1964). Fungus inoculation in nurseries. *Rep. For. Res. Inst.*, S. *Afr.* (Stencil.)

Ashton, D. H. (1956). Unpublished thesis, University of Melbourne.

Bakshi, B. K. (1966). Mycorrhiza in *Eucalyptus* in India. *Indian For.* **92**, 19–20.

Bakshi, B. K. (1967). Mycorrhiza—its role in man-made forests. *Doc., FAO World Symp. Man-made Forests, Canberra, 1967*, Vol. 2, pp. 1031–1042.

Baltaxe, R. (1967). Oral information.

Baraney, A. V. (1939). The influence of mycorrhiza on the growth and vigor of oak. *Les. Khoz.* **6**, 31–35. (Russian.)

Baraney, A. V. (1940). Experiments on the inoculation of soil with mycorrhizal fungi. *Les. Khoz.* **10**, 45–47. (Russian.)

Bassett, C. (1967). Personal communication.

Becking, J. H. (1950). Der Anbau von *Pinus merkusii* in den Tropen. *Schweiz. Z. Forstw.* **101**, 181–191.

Becking, J. H. (1965). Nitrogen fixation and mycorrhiza in *Podocarpus* root nodules. *Plant & Soil* **23**, 213–226.

Bégué, L. (1964). Le reboisement a Madagascar. *Bois For. Trop.* **94**, 3–12.

Bergemann, I. (1953). Grundlagen und Probleme der Mykorrhizaforschung in ihrer Bedeutung für die Forstwirtschaft. *Mitt. Bundesanst. Forst- u. Holzw.* No. 33.

Bergemann, I. (1955). Die Mykorrhiza-Ausbildung einiger Koniferen-Arten in verschiedenen Böden. *Z. Weltforstwirt.* **18**, 184–202.

Birch, T. C. B. (1937). A synopsis of forest fungi of significance in New Zealand. *N. Z. J. For.* **4**, 109–125.

Björkman, E. (1942). Über die Bedingungen der Mykorrhizabildung bei Kiefer und Fichte. *Symb. bot. upsaliens.* **6**, No. 2.

Björkman, E. (1949). The ecological significance of ectotrophic mycorrhizal association of forest trees. *Svensk bot. Tidskr.* **43**, 223–262.

Björkman, E. (1966). Report on problems concerning reduced growth in the second rotation of fast-growing pines in Australia. *Publ., Dep. For. Bot., Roy. Coll. For. Stockh.* **157**.

Bokor, R. (1954). A mykorrhiza-gombakkal történö talajoltások új agrotechnikai eljárása. *Erdész. Kutatás, Budapest* No. 4, 27–45.

Bokor, R. (1958). Vizgálatok a tölgyek valódi mykorrhiza-gombáinak meghatározása és az ezekkel való társulásuknak mesterséges létrehozása terén. *Erdészettud. Közl., Sopron* I, 93–118.

Bowen, G. D. (1963). The natural occurrence of mycorrhizal fungi for *Pinus radiata* in South Australian soils. *C.S.I.R.O., Div. Soils, Div. Rep.* No. 6.

Bowen, G. D. (1965). Mycorrhiza inoculation in forestry practice. *Aust. For.* **29**, 231–237.

Bowen, G. D. (1966). An annotated bibliography of Australian studies on microbial interactions with tree genera. *Aust. For.* **30**, 199–211.

Bowen, G. D., and Theodorou, C. (1967). Studies on phosphate uptake of mycorrhizas. *Pap., I.U.F.R.O., 14th Congr., Munich*, Vol. 5, pp. 116–138.

Brewer, E. F. (1967). Personal communication.

Briscoe, C. B. (1959). Early results of mycorrhizal inoculation of pine in Puerto Rico. *Carib. For.* **20**, 73–77.

British Commonwealth Forestry Conference. (1962). Pre-Conference Tour, Tanganyika, Tour Guide. (Stencil.)

Brown, J. L. (1963). Working plan for Mafuga Central Forest Reserve. Period 1964–1974. *For. Dep. Uganda.* (Stencil.)

Bryan, L. W. (1967). Personal communication.

Bryan, W. C., and Zak, B. (1961). Synthetic culture of mycorrhizae of southern pines. *For. Sci.* **1**, 123–129.

Buchholz, E. (1951). Mykotrophe Ernährung der Bäume. Sammelreferat über neuere sowjetische Forschungen. *Z. Weltforstwirt.* **14**, 51–53.

Cardoso May, L. (1967). Personal communication.

Champion, H. G., and Trevor, G. (1938). "Manual of Indian Silviculture," 374 pp. Oxford Univ. Press, Calcutta.

Chastukhin, V. Y. (1950). Methods of introduction of mycorrhizal fungi into shelterbelts. *Priroda, Moskva* **4**, 35–40. (Russian.)

Chilvers, G. A., and Pryor, L. D. (1965). The structure of eucalypt mycorrhizas. *Aust. J. Bot.* **13**, 245–259.

Clements, J. B. (1938). Trials in Nyasaland of introduced pines. *Aust. For.* **3**, 21–23.

Clements, J. B. (1941). The introduction of pines into Nyasaland. *Nyasal. agric. Quart. J.* **1**, 5–15.

Cooling, E. N. (1967a). Report of a visit to South East Asia to obtain seeds of tropical pines. *Republic Zambia, For. Res. Bull.* No. 13.

Cooling, E. N. (1967b). Personal communication.

Davey, C. B. (1967). Personal communication.

Dawkins, C. H. (1967). Personal communication.

Dominik, T. (1958). Próby przeszczepiania mikrobocenozy glebowej dzewostanów sosnowych na tereny rolne. *Prace Inst. bad. Leśn.* No. 177.

Dominik, T. (1961a). Badania nad przeszczepianiem mikrobocenoz gleb leśnych na tereny rolne. *Prace Inst. bad. Leśn.* No. 210.

Dominik, T. (1961b). Próby naturalnego wyizolowania grzybów mikrozowych z gleb rolnych i nieuzytków w okolicy Szczecina. *Prace. Inst. bad. Leśn.* No. 227.

Donald, D. G. M. (1965). A study of the history, practice and economics of forest nurseries in South Africa. *Ann. Univ. van Stellenbosch* **40A**, No. 1.

Dring, D. M., and Rayss, T. (1963). The gasteromycete fungi of Israel. *Israel J. Bot.* **12**, 147–178.

Duff, D. E. (1936). Unpublished data.

Eccher, A., and Rambelli, A. (1966). Influenza dell'inoculazione micorrizica sullo sviluppo del *Pinus radiata* D. Don. *Pubbl. Cent. Sper. agric. for., Roma* **9**, 11–22.

Eggeling, W. J. (1967). Personal communication.

F. A. O. Secreteriat. (1967). Actual and potential role of man-made forests in the changing world pattern of wood consumption. *Doc., FAO World Symp. Man-made Forests, Canberra, 1967*, Vol. 1, pp. 4–50.

Fassi, B. (1963). Die Verteilung der ektotrophen Mykorrhizen in der Streu und in der oberen Bodenschicht der *Gilbertiodendron-Dewevrei*—(Cesalpiniaceae)—Wäldern im Kongo. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 297–302. Fischer, Jena.

Fassi, B. (1967a). Recherches sur les mycorrhizes dans les pepinieres de resineux on sols agricoles. *Pap., I.U.F.R.O., 14th Congr., Munich*, Vol. 5, pp. 191–208.

Fassi, B. (1967b). Personal communication.

Fassi, B. (1969). Personal communication.

Fassi, B., and Fontana, A. (1961). Le micorrize ectotrofiche di *Julbernardia Seretii*, Cesalpiniaceae del Congo. *Allionia* **7**, 131–151.

Fassi, B., and Fontana, A. (1962). Micorrize ectotrofiche di *Brachystegia Laurentii* e di alcune altre Cesalpiniaceae minori del Congo. *Allionia* **8**, 121–131.

Fassi, B., and Fontana, A. (1966). Ricerche sulle micorrize ectotrofiche del pino strobo in vivaio. II. Micorrize di *Laccaria laccata* (Scop.) Berk. et Br. e di *Hebeloma mesophaeum* Pers. ex Fries. *Allionia* **12**, 47–53.

Fedotova, N. J. (1956). The infection of acorns with mycorrhiza for shelterbelt sowings. *Bjull. nauč.-teh. Inform. Stavr. Naučko-issl. Inst. Selsk. Khoz.* **1/2**, 13–14. (Russian.)

Fielding, J. M. (1957). Introduction of *Pinus radiata* to Australia. *Aust. For.* **21**, 15–16.

Foot, D. L. (1967). Nursery and establishment technique on the Vipya Plateau, Malawi, with special reference to the formation of a man-made pulpwood forest. *Doc., FAO World Symp. Man-made Forests, Canberra, 1967,* Vol. 3, pp. 1545–1554.

Forrest, W. G. (1965). Development of direct pine seeding techniques and the potential for use on the North Coast. *Tech. Pap., Proc. 4th Res. Officers Conf., N.S.W. For. Comm.* No. 10, pp. 29–37. (Stencil.)

Forrest, W. G. (1966). Mycorrhiza problems in pine establishment. *Proc. Refresher School Foresters, Univ. New England, Armidale, N.S.W.* (Stencil.)

Frank, A. B. (1885). Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. *Ber. dtsch. bot. Ges.* **3,** 27–33.

Freezaillah, bin Che Yeom. (1966a). The Forest Research Institute nursery. *Malay. Forester* **29,** 87–96.

Freezaillah, bin Che Yeom. (1966b). Some notes on *Pinus caribaea* Mor. grown in Malaya. *Res. Pamphl. For. Dep. Malaya* No. 54.

Freezaillah, bin Che Yeom. (1967). Personal communication.

Fries, N. (1943). Untersuchungen über die Sporenkeimung und Mycelentwicklung bodenbewohnender Hymenomyceten. *Symb. bot. upsaliens.* **6,** No. 4.

Gardner, A. (1967). Personal communication.

Gendina, S. B. (1957). The importance of seed mycorrhization to pine seedling growth. *Les. Khoz.* **10,** 46–48. (Russian.)

Gibson, I. A. S. (1963). Eine Mitteilung über die Kiefernmykorrhiza in den Wäldern Kenias. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 49–51. Fischer, Jena.

Gibson, I. A. S. (1967). Personal communication.

Gilmour, J. W. (1958). Chlorosis of Douglas fir. *N. Z. J. For.* **7,** 94–106.

Göbl, F. (1962). Das Mykorrhizaproblem bei der Zirbenanzucht. *Proc. I.U.F.R.O., 13th Congr. Vienna,* Vol. 1, No. 24, 3.

Göbl, F. (1965a). Die Zirbenmykorrhiza im subalpinen Aufforstungsgebiet. *Cbl. ges. Forstw.* **82,** 89–100.

Göbl, F. (1965b). Mykorrhizauntersuchungen in einem subalpinen Fichtenwald. *Mitt. Forstl. Bundes-Versuchsanst. Wien* **66,** 173–195.

Göbl, F. (1967). Personal communication.

Griffith, A. L., and Howland, P. (1958). Comparison of different pine soils. *Rep. E.A.A.F.R.O.* pp. 84–85.

Griffith, A. L., May, W. B., and Howland, P. (1962). Nursery experiments. *E.A.A.F.R.O. For. tech.* Note No. 15.

Griffiths, D. A. (1965). The mycorrhiza of some conifers in Malaya. *Malay. Forester* **28,** 118–121.

Groulez, J. (1967). Techniques de pepiniere de pins tropicaux au Congo-Brazzaville. *Doc., FAO World Symp. Man-made Forests, Canberra, 1967,* Vol. 3, pp. 1531–1543.

Hacskaylo, E. (1959). "Mycorrhizae of Pine in Puerto Rico." Unpublished rep. U. S. For. Serv., Washington, D. C.

Hacskaylo, E. (1965). *Thelephora terrestris* and mycorrhizae of Virginia pine. *For. Sci.* **11,** 401–404.

Hacskaylo, E., and Palmer, J. G. (1957). Effect of several biocides on growth and incidence of mycorrhizae in field plots. *Plant Dis. Reptr.* **41,** 354–358.

Hacskaylo, E., and Vozzo, J. A. (1967). Inoculation of *Pinus caribaea* with pure

cultures of mycorrhizal fungi in Puerto Rico. *Pap., I.U.F.R.O., 14th Congr., Munich*, Vol. 5, pp. 139–148.

Harley, J. L. (1959). "The Biology of Mycorrhiza," 233 pp. Hill, London.

Hatch, A. B. (1936). The role of mycorrhizae in afforestation. *J. For.* **34**, 22–29.

Hatch, A. B. (1937). The physical basis of mycotrophy in *Pinus*. *Black Rock For. Bull.* No. 6.

Holmes, W. D. (1968). Personal communication.

Horak, E. (1968). Personal communication.

Howard, J. A. S. (1956). Preliminary nursery investigations in Nyika Plateau (Nyasaland). *Emp. For. Rev.* **35**, 302–312.

Huckenpahler, B. J. (1967). Personal communication.

Imshenetskii, A. A. (ed.) (1967). "Mycotrophy in Plants" (Translated by S. Nemchonok), 362 pp. Jerusalem.

Jenik, J., and Mensah, K. O. A. (1967). Root system of tropical trees. 1. Ectotrophic mycorrhizae of *Afzelia africana* Sm. *Preslia* **39**, 59–65.

Jorgensen, J. R., and Shoulders, E. (1967). Mycorrhizal root development vital to survival of slash pine nursery stock. *Tree Plant. Notes* **18**, 7–11.

Karschon, R. (1961). Studies in nursery practice for pines. *La-Yaaram* **11**, 52–41.

Keet, J. H. (1967). Personal communication.

Kelley, A. P. (1950). "Mycotrophy in Plants," 223 pp. Chronica Botanica, Waltham, Massachusetts.

Kemp, R. H. (1967). Personal communication.

Kessell, S. L. (1923). Some observations on the establishment of pine nurseries in Western Australia. *Aust. and N. Z. Assoc. Advanc. Sci. Proc., 1923* pp. 749–751.

Kessell, S. L. (1927). Soil organisms. The dependence of certain pine species on a biological soil factor. *Emp. For. J.* **6**, 70–74.

Kessell, S. L., and Stoate, T. N. (1938). Pine nutrition. An account of investigations and experiments in connexion with the growth of exotic conifers in Western Australian plantations. *For. Bull. Perth, W.A.* No. 50.

King, N. L. (1925). *Pinus insignis* Doug. (*Pinus radiata* D. Don) in South Africa. *Union S. Africa, For. Dep. Bull.* No. 15.

King, N. L. (1934). Some notes on tree-planting with special reference to Natal. *Union S. Africa, For. Dep. Bull.* No. 29.

Klyushnik, P. J. (1952). Mycorrhizal fungi of oak. *Les. Khoz.* **5**, 63–65. (Russian.)

Kotze, J. J. (1926). *Pinus patula* Schl & Cham. Its introduction into and growth in South Africa. *S. Afr. J. Sci.* **23**, 455–466.

Krasovskaya, I. V., and Smirnova, A. D. (1959). The use of mycorrhiza at planting of oak acorns under the arid conditions of the Saratov region. *Les i Step* **2**, 29. (Russian.)

Kreisel, H. (1963). Discussion of Singer (1963a). *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 229–230. Fischer, Jena.

Laiho, O. (1965). Further studies on the ectendotrophic mycorrhiza. *Acta for. fenn.* **79**, No. 3.

Laiho, O. (1967). Field experiments with ectendotrophic Scotch pine seedlings. *Pap., I.U.F.R.O., 14th Congr., Munich*, Vol. 5, pp. 149–157.

Laiho, O., and Mikola, P. (1964). Studies on the effect of some eradicants on mycorrhizal development in forest nurseries. *Acta for. fenn.* **77**, No. 2.

Lamb, A. F. A. (1956). "Exotic Forest Trees in Trinidad and Tobago." Cov. Print., Trinidad and Tobago.

Lamb, A. F. A. (1966). Impressions of tropical pines and hardwoods in some eastern countries. *Commonw. For. Inst., Oxford* (Stencil.)

Lamb, A. F. A. (1967). Personal communication.

Lamb, A. F. A. (1968). Personal communication.

Lepoutre, B. (1964). Suite d'observations sur la régénération du cedre par taches. *Ann. Rech. for. Maroc* 7, 1–20.

Letourneux, C. (1957). Tree planting practices in tropical Asia, *FAO For. Develop. Pap.* No. 11.

Levisohn, I. (1956). Growth stimulation of forest tree seedlings by the activity of free-living mycorrhizal mycelia. *Forestry* **29**, 53–59.

Levisohn, I. (1957). Differential effects of root-infecting mycelia on young trees in different environments. *Emp. For. Rev.* **36**, 281–286.

Levisohn, I. (1958a). Mycorrhizal infection in *Eucalyptus*. *Emp. For. Rev.* 37, 237–241.

Levisohn, I. (1958b). Effects of mycorrhiza on tree growth. *Soil & Fertil.* **21**, 73–82.

Levisohn, I. (1960). Physiological and ecological factors influencing the effects of mycorrhizal inoculation. *New Phytol.* **59**, 42–47.

Lihnell, D. (1942). *Cenococcum graniforme* als Mykorrhizabildner von Waldbäumen. *Symb. bot. upsaliens.* **5**, No. 2.

Lobanow, N. W. (1960). "Mykotrophie der Holzpflanzen" (Translated by W. Rawald) 352 pp. Deut. Verlag Wiss., Berlin.

Lobanow, N. W. (1963). Die Anwendung der Mykotrophie von Holzpflanzen beim Waldanbau. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 425–440. Fischer, Jena.

Long, R. W. (1967). Personal communication.

Lowe, R. G. (1967). Personal communication.

Lundeberg, G. (1967). Raw humus as the nitrogen source for pine seedlings. *Pap., I.U.F.R.O., 14th Congr., Munich*, Vol. 5, pp. 112–115.

McComb, A. L. (1938). The relation between mycorrhizae and the development and nutrient absorption of pine seedlings in a prairie nursery. *J. For.* **36**, 1148–1153.

McComb, A. L. (1943). Mycorrhizae and phosphorus nutrition of pine seedlings in a prairia soil nursery. *Res. Bull. Iowa St. Agric. Exp. Sta.* No. 314.

McComb, A. L., and Griffith, J. E. (1946). Growth stimulation and phosphorus absorption of mycorrhizal and non-mycorrhizal northern white pine and Douglas fir seedlings in relation to fertilization treatment. *Plant Physiol.* **21**, 11–17.

McNabb, R. F. R. (1967). The Strobilomycetaceae of New Zealand. *N. Z. J. Bot.* **5**, 532–547.

McNabb, R. F. R. (1968). The Boletaceae of New Zealand. *N. Z. J. Bot.* **6**, 137–176.

Madu, M. (1967). The biology of ectotrophic mycorrhiza with reference to the growth of pines in Nigeria. *Obeche, J. Tree Club, Univ. Ibadan* No. 1, 9–18.

Marrero, J. (1962). Prácticas usadas en los viveros de pinos de Puerto Rico. *Carib. For.* **23**, 87–90.

Marx, D. H. (1966). "The Role of Ectotrophic Mycorrhizal Fungi in the Resistance of Pine Roots to Infection by *Phytophthora cinnamomi* Rands." Unpublished thesis. North Carolina State University, Raleigh, North Carolina.

May, W. B. (1953). Nursery notes to remember. *E.A.A.F.R.O. For. tech. Note* No. 1.

Melin, E. (1936). Methoden der experimentellen Untersuchung mykotropher Pflanzen. *In* "Handbuch der biologischen Arbeitsmethoden" (E. Abderhalden, ed.), Vol. XI, Part 4, pp. 1015–1108. Urban & Schwarzenberg, Berlin.

Melin, E. (1962). Physiological aspects of mycorrhizae of forest trees. *In* "Tree Growth" (T. T. Kozlowski, ed.), pp. 247–263. Ronald Press, New York.

Meyer, F. H. (1963). *Laccaria amethystina* (Bolt. ex Fr.) Besk et Br., ein zur Mykorrhizabildung an der Buche befähigter Pilz. *Ber. dtsch. bot. Ges.* **76**, 90–96.

Meyer, F. H. (1966). Mycorrhiza and other plant symbioses. *In* "Symbiosis" (S. M. Henry ed.), Vol. 1, pp. 171–255. Academic Press, New York.

Michowitsch, A. I. (1963). Untersuchungen zu Infizierung der Eiche mit Mykorrhizapilzen in der Waldsteppe und in der Halbwüste. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 441–459. Fischer, Jena.

Mikola, P. (1948). On the physiology and ecology of *Cenococcum graniforme*, especially as a mycorrhizal fungus of birch. *Comm. Inst. for. fenn.* **36**, No. 3.

Mikola, P. (1955). Artificial inoculation of mycorrhizal fungi. *Arch. Soc. Zool. Bot. Fenn. "Vanamo"* **9**, 197–201.

Mikola, P. (1965). Studies on the ectendotrophic mycorrhiza of pine. *Acta for. fenn.* **79**, No. 2.

Mikola, P. (1967). The effect of mycorrhizal inoculation on the growth and root respiration of Scotch pine seedlings. *Pap., I.U.F.R.O., 14th Congr., Munich*, Vol. 5, pp. 100–111.

Mikola, P. (1968). "The Importance and Technique of Mycorrhizal Inoculation at the Afforestation of Treeless Areas." Unpublished report. FAO. Rome.

Modess, O. (1941). Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. *Symb. bot. upsaliens.* **5**, No. 1.

Morrison, T. M. (1956). Mycorrhiza of silver beech. *N. Z. J. For.* **7**, 47–60.

Moser, M. (1956). Die Bedeutung der Mykorrhiza für Aufforstungen in Hochlagen. *Forstwiss. Cbl.* **75**, 8–18.

Moser, M. (1958a). Die Mykorrhiza, Zusammenleben von Pilz und Baum. *Umschau* No. 9, 267–270.

Moser, M. (1958b). Die künstliche Mykorrhizaimpfung an Forstpflanzen. *Forstwiss. Cbl.* 77, 1–64.

Moser, M. (1958c). Die künstliche Mykorrhizaimpfung von Forstpflanzen. II. Die Torfstreukultur von Mykorrhizapilzen. *Forstwiss. Cbl.* 77, 257–320.

Moser, M. (1958d). Der Einfluss tiefer Temperaturen auf das Wachstum und die Lebenstätigkeit höherer Pilze mit spezieller Berücksichtigung von Mykorrhizapilzen. *Sydowia* **12**, 386–399.

Moser, M. (1959). Die künstliche Mykorrhizaimpfung an Forstpflanzen. III. Die Impfmethodik im Forstgarten. *Forstwiss. Cbl.* **78**, 193–202.

Moser, M. (1962). Soziologische und ökologische Fragen der Mykorrhiza-Induzierung. *Proc. I.U.F.R.O., 13th Congr., Vienna*, Vol. 1, No. 24, 2.

Moser, M. (1963). Die Bedeutung der Mykorrhiza bei Aufforstungen unter besonderen Berücksichtigung von Hochlagen. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 407–424. Fischer, Jena.

Moser, M. (1967). Die ektotrophe Ernährungsweise an der Waldgrenze. *Mitt. Forstl. Bundes-Versuchsanst. Wien* **75**, 357–380.

Mosse, B. (1963). Vesicular-arbuscular mycorrhiza: an extreme form of fungal

adaptation. *In* "Symbiotic Associations" (P. S. Nutman and B. Mosse, eds.), pp. 146–170. Cambridge Univ. Press, London and New York.

Mukherji, S. K., and Thapar, H. S. (1961). Mycorrhizae in seven exotic conifers growing in New Forest (Dehra Dun). *Indian For.* **87**, 484–488.

Olatoye, S. T. (1966). A report of mycorrhizal investigations (Investigation 317). *Tech. Note Dep. For. Res., Rep. Nigeria* No. 33.

Oliveros, S. (1932). Effect of soil inoculation on the growth of Benguet pine. *Makiling Echo* **11**, 205–214.

Palm, B. (1930). *Pinus* and *Boletus* in the tropics. *Svensk bot. Tidskr.* **24**, 519–523.

Palmer, E., and Pitman, N. (1961). "Trees of South Africa," 352 pp. A. A. Balkema, Cape Town.

Parry, M. S. (1952). The softwood plantations of the East Transvaal and Swaziland. *E. Afr. agric. J.* **17**, 188–200.

Parry, M. S. (1953). Tree-planting in Tanganyika. I. Methods of planting. *E. Afr. agric. J.* **18**, 102–115.

Parry, M. S. (1956). Tree planting practices in tropical Africa. *FAO For. Developm. Pap.* No. 8.

Persidsky, D. J., and Wilde, S. A. (1960). The effect of biocides on the survival of mycorrhizal fungi. *J. For.* **58**, 22–24.

Peyronel, B., and Fassi, B. (1957). Micorrize ectotrofiche in una Cesalpiniacea del Congo Belga. *Atti Accad. Sci. Torino* **91**, 1–8.

Peyronel, B., and Fassi, B. (1960). Nuovi casi di simbiosi ectomicorrizica in leguminose della famiglia delle Cesalpiniacee. *Atti Accad. Sci. Torino* **94**, 1–3.

Piterbarg, R. (1967). Personal communication.

Poupon, J. (1967). Personal communication.

Procter, J. (1967). A review of nursery practice and research in Tanzania. *Doc., FAO World Symp. Man-made Forests, Canberra, 1967*, Vol. 3, pp. 1555–1567.

Pryor, L. D. (1956a). Ectotrophic mycorrhiza in renantherous species of *Eucalyptus*. *Nature* **177**, 587–588.

Pryor, L. D. (1956b). Chlorosis and lack of vigour in seedlings of renantherous species of *Eucalyptus* caused by lack of mycorrhiza. *Proc. Linn. Soc. N.S.W.* **81**, 91–96.

Pryor, L. D. (1958). How important is mycorrhiza to introduced conifers? *Aust. J. Sci.* **20**, 215–216.

Purnell, H. (1957). Notes on fungi found in Victorian plantations. III. The mycorrhizal fungi. *Plantat. tech. Pap. For. Comm. Vict.* **3**, 9–13.

Puschkinskaja, O. J., and Mischustin, E. N. (1963). Verfahren, die das Wachstum und die Mykorrhizabildung bei *Pinus* und *Quercus* unter den Bedingungen der Waldsteppenzone der UdSSR begünstigen. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 469–479. Fischer, Jena.

Pyatnitsky, S. S. (1932). Instructions for experimental shelterbelt plantations on dark chestnut soils. *Soz. lesn. Khoz. agrolesomelioratsiya* **1**, (Russian.)

Rambelli, A. (1962a). Preliminary research on mycorrhizal symbiosis in *Eucalyptus*. *Proc. I.U.F.R.O., 13th Congr., Vienna*, Vol. 1, No. 24, 27.

Rambelli, A. (1962b). Alcune indagini sulla simbiosi micorrizica in *Eucalyptus*. *Atti. Accad. Nazl. Lincei* **32**, 980–982.

Rambelli, A. (1967). Personal communication.

Rawlings, G. B. (1950). The mycorrhizas of trees in New Zealand forests. *For. Res. Notes, N. Z. For. Serv.* **1**, 15–17.

Rawlings, G. B. (1958). Some practical aspects of forest mycotrophy. *Proc. N. Z. Soc. Sci.* **3**, 41–44.

Rayner, M. C. (1927). "Mycorrhiza. An Account of Non-Pathogenic Infection by Fungi in Vascular Plants and Bryophytes," New Phytologist Reprint No. 15, 246 pp. Wheldon & Wesley, London.

Rayner, M. C. (1934). Mycorrhiza in relation to forestry. I. Researches on the genus *Pinus* with an account of experimental work in a selected area. *Forestry* **8**, 96–125.

Rayner, M. C. (1936). The mycorrhizal habit in relation to forestry. II. Organic composts and the growth of young trees. *Forestry* **10**, 1–22.

Rayner, M. C. (1938). The use of soil or humus inocula in nurseries and plantations. *Emp. For. J.* **17**, 235–243.

Rayner, M. C. (1939). The mycorrhizal habit in relation to forestry. III. Organic composts and the growth of young trees. *Forestry* **13**, 19–35.

Rayner, M. C., and Levisohn, I. (1941). The mycorrhizal habit in relation to forestry. IV. Studies on mycorrhizal response in *Pinus* and other conifers. *Forestry* **15**, 1–36.

Rayner, M. C., and Neilson-Jones, W. (1944). "Problems in Tree Nutrition," 184 pp. Faber & Faber, London.

Redhead, J. F. (1958). "A study of Mycorrhizal Associations in Some Trees of Western Nigeria." Unpublished thesis, Dept. For., University of Oxford.

Reichert, I. (1940a). Studies on mushrooms and other fungi of the forests of Palestine. I. *Boletus Boulieri* Quil. and *B. Bellini* Inzenga. *Palest. J. Bot.* (*R. Ser.*) **3**, 209–224.

Reichert, I. (1940b). Studies on mushrooms and other fungi of the forests of Palestine. II. Geography and origin of two *Rostkovites* (*Boletus*) mushrooms. *Palest. J. Bot.* (*R. Ser.*) **3**, 233–258.

Reichert, I., and Avizohar-Hershenzon, Z. (1959). A contribution to the knowledge of higher fungi of Israel. *Bull. Res. Coun. Israel* **7D**, 222–247.

Robertson, N. F. (1954). Studies of the mycorrhiza of *Pinus silvertris*. I. The pattern of development of mycorrhizal roots and its significance for experimental studies. *New Phytol.* **53**, 253–283.

Robyns, W., ed. (1935–1966). "Flora Iconographique des champignons du Congo." Bruxelles.

Roeloffs, J. W. (1930). Over kungstmatige Verjonging van *Pinus Merkusii* Jungh. et de Vr. en *Pinus Khasya* Royle. *Tectona* **23**, 874–905.

Rosendahl, R. O. (1942). The effect of mycorrhizal and non-mycorrhizal fungi on the availability of difficulty soluble potassium and phosphorus. *Proc. Soil. Sci. Soc. Amer.* **7**, 477–479.

Rubtov, S. (1964). Humusul de larice-stimulator puternik al cresterii puietilor de larice in pepiniere. *Rev. Pădurilor* **79**, 279–280.

Schramm, J. R. (1966). Plant colonization studies on black wastes from anthracite mining in Pennsylvania. *Trans. Amer. phil. Soc.* **56**, No. 1.

Shemakhanova, N. M. (1961). Effect of pure cultures of mycorrhiza-forming fungi on development of seedlings of pine and oak. *Izv. Akad. Nauk SSSR* (*Ser. biol.*) No. 3, 362–376. (Russian.)

Singer, R. (1950). Die höheren Pilze Argentiniens. *Schweiz. Z. Pilzk.* **28,** 181–196.
Singer, R. (1963a). Der Ektotroph, seine Definition, geographische Verbreitung und Bedeutung in der Forstökologie. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 223–231. Fischer, Jena.
Singer, R. (1963b). Oak mycorrhiza fungi in Colombia. *Mycopathol. Mycol. Appl.* **20,** 239–252.
Singer, R. (1964). Areal und Ökologie des Ektotrophs in Südamerika. *Z. Pilzk.* **30,** 8–14.
Singer, R., and Morello, J. H. (1960). Ectotrophic forest tree mycorrhiza and forest communities. *Ecology* **41,** 549–551.
Singer, R., and Moser, M. (1965). Forest mycology and forest communities in South America. I. *Mycopathol. Mycol. Appl.* **26,** 129–191.
Singh, K. G. (1966). Ectotrophic mycorrhiza in equatorial rain forests. *Malay. Forester* **29,** 13–18.
Singh, S., and Kumar, A. (1966). Field survey of mycorrhiza in eucalypts and pines. *Indian For.* **92,** 517–520.
Slavkina, T. I. (1963). The role of mycorrhizal fungi in the growth of conifers. *Bjull. Glavn. Bot. Sada, Moscow* **49,** 96–101. (Russian.)
Smith, N. J. G., and Pope, F. B. (1934). The association between the gasteromycete *Polysacchum* and *Eucalyptus* roots. *Trans. Brit. Mycol. Soc.* **19,** 95.
Sobotka, A. (1957). Umelá mykorrhizace semenacku dubu a borovice. *Sborn. čsl. Acad. zeměd. (Lesn.)* **3,** 503–520.
Sobotka, A. (1963). Die praktische Anwendung der Mykorrhiza bei der Aufforstung. *Mykorrhiza, Int. Mykorrhizasymp., Weimar, 1960* pp. 461–467. Fischer, Jena.
Sonn, S. V. (1955). "Report of the Scientific Expedition on the Problems of the Establishment of Shelterbelts in 1949–1952." Akad. Nauk. USSR. (Russian.)
Southern Rhodesia Forestry Commission. (1959). The raising and planting of trees on the farm. *Rhod. agric. J.* **56,** 91–105.
Stahl, W. (1967). Personal communication.
Stahl, W. (1968). Mycorrhiza fungi may be seed borne. *Inst. Forest Aust. Newsletter* **9,** 11.
Stepanov, H. H. (1932). "Afforestation of the Steppes." Moscow. (Russian.)
Stephens, E. L., and Kidd, M. M. (1953a). "Some South African Edible Fungi." Longmans, Green, Cape Town.
Stephens, E. L., and Kidd, M. M. (1953b). "Some South African Poisonous Fungi." Longmans, Green, Cape Town.
Stephens, R. P. (1931). Unpublished data.
Stoeckeler, J. H., and Jones, G. W. (1957). Forest nursery practice in the Lake States. *Agric. Handb. U.S. Dep. Agric.* **110.**
Stoeckeler, J. H., and Slabaugh, P. E. (1965). Conifer nursery practice in the prairie-plains. *Agric. Handb. U. S. Dept. Agric.* **279.**
Stone, E. L. (1950). Some effects of mycorrhizae on the phosphorus nutrition of Monterey pine seedlings. *Proc. Soil. Sci. Soc. Amer.* **14,** 340–345.
Streets, R. J. (1962). "Exotic Forest Trees in the British Commonwealth," 765 pp. Oxford Univ. Press (Clarendon), London and New York.
Stuart-Smith, A. M. (1953). "Pine Nursery Technique, with Special Reference to the Techniques Employed in the Uganda Protectorate and with Suggestions,

as How These May be Improved." Unpublished thesis, Dept. For., University of Oxford.

Swabey, C. (1967). Personal communication.

Takacs, E. A. (1961a). Algunas especies de hongos formadores de micorrizas en árboles forestales cultivados en la Argentina. *Rev. for. argent.* **5**, 80–82.

Takacs, E. A. (1961b). Inoculación de especies de pinos con hongos formadores de micorrizas. *Silvicultura, Uruguay* **15**, 5–17.

Takacs, E. A. (1964). Inoculación artificial de pinos de regiones subtropicales con hongos formadores de micorriza. I. Formacion de micorrizas ectotroficas en *Pinus taeda* L. con *Scleroderma vulgare* Horn. *Idia, Suppl. For.* **2**, 41–44.

Takacs, E. A. (1967a). Producción de cultivos puros de hongos micorrizógenos en el Centro Nacional de Investigaciones Agropecuarias, Castelar. *Idia, Suppl. For.* **4**, 83–87.

Takacs, E. A. (1967b). Personal communication.

Titze, J. F. (1967). Personal communication.

Trappe, J. M. (1962a). Fungus associates of ectotrophic mycorrhizae. *Bot. Rev.* **28**, 538–606.

Trappe, J. M. (1962b). "*Cenococcum graniforme*—its Distribution, Ecology, Mycorrhiza Formation, and Inherent Variation." Unpublished thesis, University of Washington. Seattle, Washington.

Trappe, J. M. (1964). Mycorrhizal hosts and distribution of *Cenococcum graniforme*. *Llyodia* **27**, 100–106.

van Alphen de Veer, E. J., *et al.* (1954). Artificial regeneration of *Pinus merkusii*. *Proc. 4th World For. Congr., 1954* Vol. III, pp. 565–572.

van Suchtelen, M. J. (1962). Mykorrhiza bij *Pinus* spp. in de tropen. *Med. Landb-Hogesch. Gent.* **27**, 1104–1106.

Vega Condori, L. (1962). Introduction of conifers to different ecological zones of Costa Rica and the effect of mycorrhiza on their initial growth. Summary of Seminar, Centro Tropical de Investigación y Ensenanza, Turrialba. (Stencil.)

Vega Condori, L. (1964). Effect of mycorrhizae on the initial growth of tropical conifers. *Turrialba* **14**, 151–155.

Volkart, C. M. (1964). Formación de micorrizas en pinos centro-americanos bajo condiciones controladas. *Turrialba* **14**, 203–205.

Voznyakovskaya, Y. M., and Ryžkova, A. S. (1956). A technique of mass propagation of mycorrhizal fungi. *Bjull. Nautsh-Techn. Inform. Mikrobiol.* **1**, (Russian.).

Vozzo, J. A. (1967). "Inoculation of Pine with Mycorrhizal Fungi in Puerto Rico." Unpublished thesis, George Washington University, Washington, D. C.

Vyssotsky, G. N. (1902). Mycorrhiza of oak and pine tree seedlings. *Lesoprom. Vestnik* **29**. (Russian.)

Vyssotsky, G. N. (1929). A reminder to steppe foresters on mycorrhiza. *Les. Khoz.* **10/11**, 19–44. (Russian.)

Wadsworth, F. H. (1967). Personal communication.

Wahl, I., and Reichert, I. (1953). The die-back of Aleppo pines and its relation to mycorrhiza development. *Palest. J. Bot. (R. Ser.)* **8**, 196–204.

Wakeley, P. C. (1954). Planting the southern pines. *Agric. Monogr. U. S. Dept. Agric.* **18**, 233 pp.

Waterer, R. R. (1957). Nursery practice for conifers in Kenya. *Techn. Order Kenya For. Dep.* No. 17.

Weck, J. (1961). Tatsachen und Probleme einer Weltforstwirtschaft. *In* "Weltforstwirtschaft und Deutschlands Forst- und Holzwirtschaft" (J. Weck and C. Wiebecke, eds.), pp. 1–74. BLV, Munich.

White, D. P. (1941). Prairie soil as a medium for tree growth. *Ecology* **22**, 398–407.

White, J. K. (1967). Personal communication.

Wilde, S. A. (1944). Mycorrhizae and silviculture. *J. For.* **42**, 290–291.

Wilde, S. A. (1954). Mycorrhizal fungi: Their distribution and effect on tree growth. *Soil Sci.* **78**, 23–31.

Wilde, S. A., and Persidsky, D. J. (1956). Effect of biocides on the development of ectotrophic mycorrhizae in Monterey pine seedlings. *Proc. Soil. Sci. Soc. Amer.* **20**, 267–268.

Wimbush, H. (1967). Personal communication.

Worley, J. F., and Hacskaylo, E. (1959). The effect of available soil moisture on the mycorrhizal association of Virginia pine. *For. Sci.* **5**, 267–268.

Young, H. E. (1940). Mycorrhizae and the growth of *Pinus* and *Araucaria*. *J. Aust. Inst. Agric. Sci.* **6**, 21–31.

Zak, B., and Marx, D. H. (1964). Isolation of mycorrhizal fungi from roots of individual slash pines. *For. Sci.* **10**, 214–222.

Zerling, G. I. (1958). Die Mykorrhiza der Lärche und ihre Wirkung auf das Wachstum und den Zustand der Sämlinge in Karbonat-Tschernosem-Böden des Transvolga-Gebietes. (Translated by M. Moser.) *Mikrobiologiya* **27**, 450–459.

Zerling, G. I. (1960). Die Mykorrhizabildung bei Lärche unter den Bedingungen des Transvolga-Tschernosems und ihre Stimulierung. (Translated by M. Moser.) *Mikrobiologiya* **29**, 401–407.